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Where is Federico Alvarez Rojas?

LAST year (*Nature* 263, 452) we reported on the plight of Maximo Pedro Victoria, an Argentinian nuclear engineer imprisoned and brutally treated for no more obvious reason than that he, as a civilian engineer, had been in dispute several years previously with military scientists over the fuel for a new reactor. It seemed that now the military was all-powerful in Argentina, some old scores were being settled; Dr Victoria, who had been a member of the Board of Directors of the National Institute of Technology, had to be taught a lesson which included being beaten up and losing some of his teeth.

Late in October, Dr Victoria was freed; 'your husband must be a real angel to be let out of gaol', a military officer told his wife. He now lives in Belgium. Maybe, we naively thought, this is a turning point and the Argentinian authorities are beginning to let up on the extremely repressive way in which they have been dealing with subversives, real or imagined. Not so; a report just issued by Amnesty International of an international mission to Argentina (Amnesty Dept A, 55 Theobald's Road, London WC1; £1.00) makes it abundantly clear that despite President Videla's assertion that 'there are no Argentines detained for their political opinions', there could be several thousand political prisoners, most not tried or even charged, and some subjected to unspeakable brutalities.

A particularly alarming aspect of the present troubles is the number of people who disappear or are abducted. Estimates of numbers vary widely but since the military coup in March 1976, at least 2,000 have disappeared; in the last week of May 1976 relatives filed a total of 200 writs of *habeas corpus* in Buenos Aires alone. What happens to these people? Representatives of the Argentinian government pointed out to the mission that they might have chosen to go underground or to emigrate, or they may have been killed in a clash with security forces. This looks like only a part of the story; there seems hardly any doubt that many of those missing were taken into custody and that many of the remainder were killed by right-wing terrorist groups which seem to operate without much hindrance from the government. Indeed, recently the Minister of Foreign Affairs, in comparing left-wing guerrillas with microbes on the social body, described reprisals groups as antibodies

which would disappear when the microbes were eliminated.

Among the several hundred whom Amnesty list as having disappeared are several scientists. These include Federico Alvarez Rojas, a physicist at the National Atomic Energy Commission (CNEA), and his wife Hilda Leikis, a computer programmer; Roberto Ardito, an electrical engineer at CNEA, and his wife and children; Ricardo Chidichimo, an Air Force metallurgist; and Antonio Misetich, a CNEA physicist with an international reputation. The case of Federico Alvarez Rojas will illustrate the problem.

Senor Alvarez Rojas, 33 years of age, has worked at CNEA since 1968, most recently as director of the X-ray diffraction laboratory; his wife worked as a programmer at Bairesco S.A. (Burroughs). His father, also Federico Alvarez Rojas, was in Britain for some time as a British Council Scholar and worked in the Soil Mechanics Division of the Building Research Station, Garston. Senor Alvarez Rojas Jr and his wife were abducted by an armed group in the early hours of 1 October 1976; their three children were left behind. Since then vigorous efforts to find them have been made by his father who has issued *habeas corpus* writs and appealed to the authorities, the church and the Red Cross without success. Efforts made through American and British diplomatic channels have been unsuccessful.

What can we do? We have a responsibility as scientists to be concerned about illegal actions against any member of the scientific community whether the person in question is known to us or not. As the recent Council for Science and Society report on scholarly freedom and human rights put it, science is particularly vulnerable to outside interference, and it is clear that there is outside interference of an exceedingly ugly kind in the national laboratories of Argentina. The rapid deterioration in conditions caused by the recent events is soon going to leave the country, in the words of one despairing correspondent, 'inhabited only by labourers and murderers'. Let us hope that this message gets through to the president, General Jorge Videla, to whom it is still worth writing at Balcarce 50, 1064 Buenos Aires, to enquire about the fate of fellow members of the scientific community. □



A fishy business

C. E. Purdom and A. Preston assess the state of fish farming and its future promise

FISH farming is arousing widespread interest on the basis of a variety of concepts, some of which are not valid. There is such confusion about the motives of fish farming research that it is necessary to define clearly the role that this form of animal husbandry has within the context of UK food supplies.

Throughout the world, fish farming takes many forms but there are three basic categories:

- sea ranching: fish are reared for release into the sea as juveniles, where they subsequently grow to marketable size;
- extensive production using natural productivity;
- intensive farming which relies on supplied feeds.

Sea ranching has strict limitations, being feasible only where the natural tendency of the fish is to return to the point of release when they are fully grown. Salmon and sturgeon in the United States and the Soviet Union respectively are farmed in this way. There seems little immediate prospect that private enterprise will be stimulated by the vision of a valuable investment swimming off to distant waters.

Extensive cultivation is carried out basically by warm water pond farming. It is by far the most widespread of fish farming systems on a global basis and is found in most areas where space, climate and a local market justify it. Carp (*Cyprinus carpio*) is the archetypal species in pond farming, but a quite spectacular growth occurred in catfish (*Ictalurus punctatus*) farming in the southern states of the United States during the 1960s and there has been recent progress with several other species of fish in fresh and brackish waters in south-east Asia.

Pond fish farming depends upon natural productivity and, like any other form of extensive animal husbandry, benefits from pest control, the use of fertilisers and the supply of supplementary feeds. Its productivity is governed by management practices and, of course, by the natural features of the farm. Natural productivity alone is low, perhaps a few hundred kilograms per acre per year at best. Fertilisation and other water management practices can double this but big dividends come only from the use of supplementary feeds. Here is a snag: fish are not efficient users of food

materials other than animal protein and fat. Occasional reference is made to the desirability, in a protein saving context, of rearing herbivorous fish. The fact that such species require over 100 kg of feed to make 1 kg of flesh, however, puts them way below the performance of farm ruminants.

Intensive farming is almost wholly artificial. Fish are held in small containers which are commonly circular tanks, linear raceways or net enclosures in tidal waters. Very high stock densities are maintained with a large flow-through of water. At fish densities up to 100 kg m⁻³ all the nutritional requirements must be met in prepared feeds which must contain high levels of animal protein, 40–45% dry weight, moderately high lipid content and vitamin supplements. Such feeds are expensive, but the rationale of intensive farming is to produce high quality protein from low quality protein (quality being defined in terms of acceptability for human consumption). Feed protein is converted into fish protein with an efficiency of about 30% and intensive farming is therefore a net user of protein. The fish most commonly farmed this way are salmon and trout, in fresh or salt water, but large quantities of yellow-tail, a fully marine species, are farmed intensively in Japan.

No cheap protein

It is abundantly clear that fish farming is not a means of producing cheap protein. Whenever fish are farmed, they are high quality, high priced foods. A further view of fish farming is that it is an alternative, or a meaningful supplement, to the yield from sea fisheries. This idea is also invalid on the grounds that the two spheres of activity are of widely differing scales. UK fish landing approach one million tonnes per year: currently, fish farming contributes 2,500 tonnes per year. Assuming a ten-fold increase during the next five years, which is both worthwhile and probable, the scale of fish farming will still be totally inadequate to accommodate the fluctuations in the yield from sea fisheries, which can follow from political or management decisions or from natural causes: these may be measured in hundreds of thousands of tonnes. However, for individual species of fish, for which landings are low, for example turbot (700 tonnes UK landings in 1975) or sole (1,300 tonnes UK landings in 1975), there is some prospect of significantly



Turbot: no pigment problem

adding to supplies by farming.

Fish farming in the UK probably will be based on intensive systems for the supply of specific types of fish suitable for these systems and for which a current demand exists. The two species likely to fulfil these requirements are rainbow trout (*Salmo gairdnerii*), which constitutes the bulk of present farm production, and turbot (*Scophthalmus maximus*), which has been researched intensively over the past five years and is currently under pilot scale commercial production.

Research on plaice, sponsored by the Ministry of Agriculture, Fisheries and Food (MAFF) began in the 1950s. This species is no longer thought suitable for cultivation because it is relatively abundant around the UK, has a fairly stable population, and is not a good enough converter of food to sustain an economically viable industry. After much background research, therefore, attention was turned to sole and turbot which are prime fish of high value and in short supply. Mass rearing techniques were quickly established for sole along the lines developed for plaice. Little progress was made with on-growing (that is, rearing hatched fish to marketable size and sexual maturity), because the hatchery-reared juvenile fish were difficult to wean onto prepared diets. The sole is a quiet feeder with nocturnal, browsing habits—the introvert of the flatfish families. Further research in MAFF laboratories was restricted to the weaning phase. Turbot was assessed first in terms of its on-growing capabilities. It passed the test, being voracious, a good protein converter (achieving levels up to 50%), and robust and docile towards anything it could not swallow. Growth rate is then dependent on temperature.

Hatchery techniques

Following the satisfactory outcome of these trials, using small fish collected from the beaches, efforts were made to develop a hatchery technique to raise juveniles from eggs. Plaice and sole larvae were satisfactorily grown

on a diet of *Artemia* nauplii. Turbot, however, were too small initially to feed on nauplii but they could feed on natural plankton, mussel veligers and the rotifer *Brachionus plicatilis*, the latter being the most suitable. After a few days feeding on rotifers, turbot larvae accepted nauplii but the problems did not end there. Metamorphosed fish were reared for the first time in 1972; about 100 were produced from many thousands of eggs. Steady annual improvements in empirical techniques have since been made, and survival rates up to 25% are now feasible.

Solving the early feeding needs of the larvae, at least at an empirical level, did not remove all the obstacles to successful rearing of turbot. A number of minor problems, 'dropsy' in young larvae, a later lack of swim-bladder development and atypical pigment formation at metamorphosis, mostly diminished with increasing experience. A major problem, however, was that, unlike plaice and sole, turbot larvae were not satisfied for long on a diet of *Artemia* nauplii. Progressively larger *Artemia*, grown on unicellular algae, were required by the fish as they grew larger themselves. A weaning process was developed whereby the larvae were switched to prepared foods by using specially designed weaning tanks which kept inanimate food particles moving but did not sweep the fish down the overflow. Rearing turbot larvae is now almost routine but it still requires sophisticated laboratory facilities and further research is needed to clarify scientific aspects and to simplify systems for commercial use.

Sole have similarly been weaned from *Artemia* to prepared diets but for rather different reasons. Earlier work with sole relied on a succession of live foods but these are not suitable for commercial use and they not only delay the problem of weaning but also intensify it: the luxury of live foods appeared to be habit forming. A clear demonstration that sole is suitable for domestication still remains to be made.

Coping with seasonal spawners

A problem with turbot, as with most fish, is that spawning is seasonal. This restricts management options in commercial farming and it also limits the pace at which research can proceed. Most seasonal spawners have light or temperature regulated hormonal clocks and in theory can be persuaded to spawn out of season by the correct manipulation of whatever parameter is appropriate. On the basis of successful work with dab, trials were set up to influence the spawning pattern of turbot, which normally spawns in mid-summer. Spawning has been brought

forward to March in one series and further trials are aimed at delaying spawning until early winter. The indoor work of hatching and larval rearing could then be completed by spring when the juvenile fish could make use of increasing temperatures outdoors.

Work with flatfish has been aimed at creating a new form of fish farming. Recent commercial investment in this area is a measure of its success and has introduced the provision of advice and assistance to industry through research and development programmes. The growth of trout farming led to a similar demand for R&D programmes and, after a recent review, it was planned that all the MAFF's fishing farming research, except work on infectious diseases, should be located at the Fisheries Laboratory, Lowestoft.

The trout research programme is divided into water management, genetics and feeding strategies. Water is clearly a limiting factor in trout farming and in simple systems about 20,000 gallons are used for each pound of fish produced. But the first limiting factor is oxygen. Water-saving systems employing oxygen or air are under development by industry. Removing the limitation of oxygen increases the levels of carbon dioxide, ammonia, nitrite and nitrate. Limited research on the effects of these and other water quality factors is planned but the principal MAFF investigation will be on the use of sea water for farming trout. This offers immediate scope for large scale increases in production.

Rainbow trout are not naturally anadromous but are successfully reared in sea water in some parts of the world. Smolting does not occur, as in salmon and migratory trout, but the ability of rainbow trout to withstand transfer to sea water increases with their size. Preliminary trials suggest that 30–90 g is the range over which fish may be acclimatised but there is evidence that

age may be important and that domesticated strains vary in their tolerance of sea water. Clearly it is important to establish the earliest possible transfer to sea water where portion-sized fish of around 250 g are produced, but for larger fish, for which there is a growing market, the need is not so marked.

The genetic programme is based on the fact that several individual strains of rainbow trout already exist. A number of these strains have been imported with the objective of developing disease-free broodstock for subsequent trials both of the characteristics of the strains themselves and of cross-breeds. Electrophoretic analysis of enzyme polymorphisms suggests that some of the strains are inbred.

Strains differ in the time at which they spawn and it may be possible with trout to supply eggs throughout the year without recourse to environmental manipulation—more difficult for trout than for turbot on account of their relatively low fecundity and the need for large numbers of broodstock. Other genetic aims include selection for larger egg size and for late maturity.

Feeding is by far the most cost sensitive area of trout farming but despite a long history of domestication, there is little standardisation of feeding strategies. Current practice in the UK is to use pelleted feeds but in other countries, much reliance is placed on the use of cheap trash fish, particularly in sea water culture. Routine use is made of such materials within Ministry work but detailed nutritional and logistic assessment remains to be made.

Fish farming as a food producing industry has no other commitment but to operate in a cost-effective way. MAFF research has established a basis for turbot farming and continues to explore this and rainbow trout farming in order to supply industry with scientific advice. □



Cages of plaice and turbot in the North Channel

USA

Start from the middle

The results of a year-long study of nuclear power by a distinguished panel were published last week. Colin Norman reports from Washington

In a report likely to have a significant impact on nuclear policy in the United States, and perhaps elsewhere, a panel of distinguished scientists and economists has urged the Carter Administration to bar domestic reprocessing of spent reactor fuel, and to curtail drastically the present crash effort to develop a commercial breeder reactor. The economic justifications advanced for those two programmes are dubious at best, the panel said, and to proceed apace with either of them would seriously hamper attempts to curb the proliferation of nuclear weapons.

With the Carter Administration now in the throes of putting together a comprehensive energy policy, to be unveiled on 20 April, the report* couldn't have come at a more opportune moment. In fact, the panel's conclusions are in harmony with some of President Carter's campaign statements, and it is generally expected that the recommendations will be reflected strongly in Carter's energy policy. In any case, the report is said to have gone down well in the White House, and Carter even took time last week to meet with all 21 panel members to discuss their recommendations.

The product of a year-long study sponsored by the Ford Foundation, the report is an attempt to examine and stitch together the economic, political and social arguments which have been raging over nuclear power in the past few years. The effort was initiated, according to the Ford Foundation's President, McGeorge Bundy, because of "a widespread feeling that this debate was suffering from a shortage of disinterested analysis", and the foundation therefore chose as members of the panel prestigious individuals who were not lined up on either side of the debate. Like the report of the Royal Commission on Environmental Pollution in Britain, the panel's conclusions have therefore gained some authority from the fact that the panel started from a middle-of-the-road position.

The panelists were mostly liberal academics many of whom have been

active in arms control issues, and they include two top officials in the Carter Administration—the Secretary of Defense, Harold Brown, and Joseph Nye, deputy Under-Secretary of State (Brown was President of Caltech when he served on the panel and Nye was professor of government at Harvard). Other members included John Sawhill, former Administrator of the Federal Energy Agency, Wolfgang Panofsky, director of the Stanford Linear Accelerator Center, Kenneth Arrow, a Nobel prizewinner and professor of economics at Harvard, and Richard Garwin of IBM. The chairman of the panel was Spurgeon M. Keeny, a senior official of the MITRE Corporation, a think-tank which coordinated the effort.

With such an impressive list of authors, it's not surprising that the study has attracted considerable attention. It should be noted, however, that contrary to some interpretations, it is not an anti-nuclear document. Far from it. Though the panel challenges many of the chief arguments put forward by the nuclear industry, and opposes some programmes which the industry believes are crucial for its long-term prospects, the report nevertheless acknowledges that uranium is likely to be an important source of energy for several decades and it suggests that in some respects nuclear power may hold a slight edge over alternative energy sources.

The panel takes issue with conventional nuclear wisdom on two points fundamental to the nuclear debate, however. It argues that, in the United States at least, although nuclear power seems to hold a slight economic advantage over coal, "the choice is so close and the uncertainties sufficiently large that the balance could easily shift to increase or eliminate" that advantage. Similarly, adverse health and social effects associated with nuclear power are likely to be less than those associated with coal, but "the range of uncertainty in social costs is so great that the balance between coal and nuclear power could be tipped in either direction".

That is a far cry from the industry's contention that the atom would supply significantly cheaper and cleaner energy than alternative sources, but it leads the panel to suggest that the United States should maintain a mix between coal and nuclear power. The recommendation is really an acknowledgment of what is likely to happen anyway.

The panel is, however, much less equivocal in its conclusions concerning plutonium. Citing nuclear proliferation as "the most serious danger associated with nuclear power", the panel urges the Carter Administration to renounce domestic reprocessing of spent reactor fuel and recycling of plutonium. "If a decision to postpone this technology indefinitely is articulated and carried out effectively", the panel argues, "it can have a major influence on the assessment of costs and benefits of reprocessing and recycle by other countries that are, or soon will be, facing similar decisions. Conservatively, a US decision to go ahead with reprocessing . . . would accelerate worldwide interest in the plutonium fuel cycle and undercut efforts to limit nuclear weapons proliferation".

In any case, the panel argues that reprocessing is difficult to justify even on economic grounds. The nuclear industry has long argued that reprocessing and plutonium recycle will be needed to stretch out dwindling supplies of uranium and to pave the way for plutonium-producing breeder reactors, but the panel takes issue with the industry's chief assumptions. "Our review convinces us that current official estimates of uranium reserves and resources substantially underestimate the amounts of uranium that will be available at competitive costs", it notes, and suggests that "there will be enough uranium at costs of \$40 (1976 dollars) per pound to fuel light water reactors through this century and, at costs of \$40 to \$70 per round, well into the next century". Moreover, the panel points out that the estimated costs of reprocessing have escalated rapidly, with the result that "any net economic benefit during this century is questionable".

The panel therefore urges Carter to short-circuit a major review by the Nuclear Regulatory Commission of whether reprocessing should be permitted by announcing that the United States will defer use of the technology indefinitely. In fact, Carter promised during his election campaign that he would "seek to withhold authority for commercial reprocessing until the need for, the economics and safety of this technology is clearly demonstrated", and the panel is therefore essentially urging him to keep his campaign pledge.

As for the breeder reactor programme, the panel again takes issue with the industry's economic arguments, suggesting that "the early economic potential of the breeder has been significantly overstated". The

**Nuclear Power Issues and Choices* (Baling Publishing Co., Cambridge, Mass. \$6.95.)

report also notes that a breeder programme would involve full-scale commitment to the plutonium cycle with its attendant proliferation problems. In spite of those drawbacks, however, the panel doesn't recommend complete abandonment of the programme, but suggests instead that the effort should be sharply reduced in priority and be pursued only as "insurance" against the possibility of very high future energy costs or the failure of efforts to develop alternative energy sources.

The effort to develop a commercial breeder reactor has long enjoyed pride of place as the federal government's most expensive and most controversial energy research and development programme. The expected cost of the programme has soared from \$2,000 million to \$12,000 million in the past few years, and the projected date for introduction of commercial breeder reactors has slipped from the late 1980s to the mid 1990s. A small test reactor, the Fast Flux Test Facility, is now nearing completion in Washington State and it is expected to start operations in 1980. The plan is to follow that with a 380 MW demonstration reactor, construction of which was expected to begin on the Clinch River in Tennessee this year, with operation scheduled for 1983. The next step would be a large prototype reactor, funded chiefly by electricity utilities, with construction scheduled to begin in 1981 and operation in 1988. A decision on whether to

proceed from the prototype to a full-scale commercial programme would be made in 1986.

The Ford Foundation study suggests that the overlapping, crash programme leading to a decision on commercialisation as early as 1986 is unwarranted, and it recommends that the effort be completely revamped with more emphasis placed on technology development. In particular, the panel recommends that the Clinch River plant be abandoned, and it suggests that the decision on commercialisation can safely be postponed beyond the end of the century.

Again, that recommendation meshes neatly with Carter's campaign pledge to reduce the breeder programme to a "relatively low priority, possibly multinational effort". Last month, moreover, Carter recommended that nearly \$200 million be cut from the Ford Administration's budget request for the breeder programme, and he ordered the Energy Research and Development Administration to undertake an intensive study of the need for and timing of the effort. That study is now under way, for ERDA established last month a committee containing some of the breeder programme's most vocal supporters and critics, and asked it to come up with recommendations before Carter's 20 April energy statement.

The Ford Foundation panel also urges the Carter Administration to take a number of other steps to reduce

pressures leading to nuclear proliferation. In particular, it suggests that "the United States must have a clear policy on its long-term role in providing enriched fuel to both domestic and foreign nuclear power programs". The government should retain ownership of uranium enrichment facilities, the panel recommends, thereby reversing the Ford Administration's goal of turning enrichment plants over to private industry. The United States should also ensure that there is ample enrichment capacity in the 1980s and 1990s to meet international commitments, for the panel suggests that an assured fuel supply would reduce the incentive for other countries to develop their own enrichment and recycling facilities.

Finally, the panel recommends that the government should proceed promptly with plans to test the feasibility of disposing of nuclear wastes in salt beds and other stable geologic formations. Decisions on waste disposal have been continually deferred, pending introduction of reprocessing, a fact which has led to the widespread belief that reprocessing is an essential pre-requisite for permanent disposal (a belief which is written into legislation in Germany requiring that reactor wastes be reprocessed before disposal). The panel notes, however, that "spent fuel can be disposed of directly, and probably at costs comparable to those for reprocessed wastes". □

SERI site selected

THE Energy Research and Development Administration (ERDA) last week conferred a much sought after plum on the State of Colorado. It selected a site in the Denver suburbs for the long-awaited Solar Energy Research Institute (SERI), a facility which could develop into the leading solar energy research centre in the United States.

Altogether nineteen organisations in 16 different states had been vying for SERI during the past year; the winner is the Midwest Research Institute, a St Louis-based research organisation, which teamed with Colorado to offer a proposal.

To help soften the blow for some of the unlucky contenders, ERDA also announced last week that it will establish three regional solar energy research centres in New England, the Southeast, and the upper Midwest. The regional centres are expected to be run by consortia including representatives of several state governments, though their full role in the overall solar effort has yet to be determined.

SERI was called for in the Solar Energy Research, Development and Demonstration Act, a bill passed in 1974 which directed ERDA to establish a national facility and to seek the advice of the National Academy of Sciences on its role and scope. It was generally anticipated that SERI would be a large facility along the line of ERDA's nuclear laboratories.

The Academy furthered that expectation with a report recommending that SERI should be a major operation responsible for all phases of solar energy research including photovoltaics, fuels from biomass, wind power and so on. SERI should have a large budget, amounting to about \$50 million by 1980, the Academy suggested. With very few large scientific projects up for grabs these days, such a big-league enterprise immediately generated a lot of attention and proposals came flooding into ERDA.

Early last year, however, ERDA, under pressure from the Office of Management and Budget, opted for

something rather less grandiose. It announced that SERI would start small—a budget of about \$5 million for its first year—and a decision would be made early in the 1980s on whether it should be expanded to the sort of facility the Academy had in mind. Even that scaled-down version attracted 20 separate bids, however, and ERDA shortlisted 19 of them for further study.

In announcing the selection of the Midwest Research Institute/Colorado proposal, ERDA Acting Administrator Robert Fri said last week that SERI will start with a budget of \$4–6 million for its first year, and a staff of about 75 professionals. It will cover all aspects of solar energy, but initially its functions will be mostly concerned with assessments, analyses, information dissemination, education and consultation. Its research and development activities will build up more slowly, and ultimately it could be moved to its own 300-acre site nearby at the foot of the Rocky Mountains.

Colin Norman

BRITAIN

● When the UK Department of Energy (DEN) published the report of its Harwell-based Energy Technology Support Unit (ETSU) on solar energy in the United Kingdom last month, it simultaneously announced the launching of a £3.6 million programme of research and development over the next four years. Full details of how the money would be allocated were not published, but now the government has given a breakdown by department of the overall annual expenditure in the various fields of solar R&D (see table, below).

The details emerged last week at a solar energy workshop organised by the government's Inter-Departmental Steering Committee on Solar Energy R&D; it was attended by some 200 people from national and local government, public and private industry, research institutes and corporations, and universities and polytechnics.

Britain's solar energy effort involves expenditure of some £6 million over four years and stretches beyond the DEN programme, which mainly involves supporting commercial exploitation of solar water and space heating technologies and £600,000 of backing for biological conversion, in which NERC, ARC and MAFF have all shown interest. ETSU meanwhile is to establish this year a register of current R&D on new energy sources and energy conservation relevant to the DEN programmes, but to preserve confidentiality the register will not be published.

The Department of the Environment (DOE) work focuses principally on solar water and space heating too, mainly through its Building Research Establishment. The Department of Industry (DOI) is involved in photovoltaic conversion and supports a solar cell group at the Royal Aircraft Establishment's Space Department, a representative from which last week urged greater co-ordination of support from the SRC and DOI.

The DOI now has an information officer attached to the Solar Energy Unit based at University College,

Cardiff. As for the attachment of an Energy Research Support Unit to the SRC's Rutherford Laboratory, to provide assistance and support for energy research in universities and polytechnics, this was welcomed by the outgoing chairman of the UK section of the International Solar Energy Society (UK-ISES), who strongly urged devotion by universities to development now that scientific principles were known.

Britain's international involvement is mainly through the EEC, through which international collaboration is being sought in the particular field of thermal power generation, although last week one official from the DOE's Directorate of Research Requirements personally questioned whether UK efforts in this area ought to be concentrated on large-scale plants instead of "something smaller to meet export opportunities".

EEC expenditure amounts to some £320,000 a year in direct support of work at its Joint Research Centre, and £1.75 million in indirect partial support of projects elsewhere.

● Predictably, the first British response to the saccharin developments in North America was a parliamentary question in the House of Commons. The Ministry of Agriculture, Fisheries and Food sees no reason why Britain should ban the sweetener as well, but the data and results of the research are to be examined by expert committees before any action is taken.

The stir caused by the saccharin revelations added poignancy to the publication last week of *Why Additives? The Safety of Foods*, a booklet devised and edited by the British Nutrition Foundation, which is closely linked with the food industry. Many of the scientists who wrote it are also associated with the industry. Although meant to reassure the big public, the views expressed are not as suspect as many bromides which emanate from a nervous industry since, as the booklet explains, the use of additives in this country is strictly

controlled, compounds are constantly tested, and use is based on a permitted list.

The booklet lists saccharin among additives requiring further evidence on safety and toxicology, but stresses that 80 years of widespread use is a good indication that it is "harmless", particularly as regular users include diabetics who see their doctors frequently. The main message is clear: additives make food safer, prolong storage life, allow bulk manufacture or are added for nutritional reasons.

Among additives meant to please, rather than prevent food poisoning or outrageous cost, are colourings. Man-made colourings have been more closely investigated than nature's chemical colours, but the benefits they offer are often purely aesthetic, though attempts to avoid colouring can be commercially disastrous. A former member of the Government's Food Additive and Contaminants Committee (FACC) suggested last week that colouring matter should not be added to infant foods or fresh fruit, meat or vegetables. The case of infant food is currently under review.

Anxiety also exists about the use of nitrites as antibacterial preservatives in bacon and other meat products. The authors of the booklet suggest that the risk of poisoning from botulism is a much greater hazard than any carcinogenic danger, but only experimental evidence can confirm this.

● The stark features about income and expenditure figures of the Imperial Cancer Research Fund (ICRF)—apart, that is, from their sheer size—are that the income all comes from legacies, subscriptions and donations, and the expenditure is channelled into research. Thus, according to the 1976 report published last week, just 3.3% of expenditure in 1975–76 went on administration, and 2.6% to appeals and publicity. Of the remainder—all £8 million of it—£3.9 million was spent directly on research, £2.8 million augmented the ICRF endowment and £1.3 million went on "extraordinary items".

The ICRF chairman, Professor Sir Eric Scowden, says in his report that "far-reaching changes are imminent" at the Lincoln's Inn Fields laboratories. Michael Stoker, the Director of Research, intends to retire in September 1979, and Renato Dulbecco, the Deputy Director, retires this September; the ICRF Scientific Advisory Committee is considering the future of "these vital appointments".

UK government involvement in solar R & D (£'000 p.a. on average)¹

	Solar water and space heating	Insolation data	Photochemistry/Photobiology	Photovoltaic conversion	Total
DEN	700	50	150		900
DOE	125				125
DOI	15 ²			215	230
Met. Office		50			50
SRC	35		90 ³	25	150
Total	875	100	240	240	1,455

¹The figures are a rough indication of present allocations, and give an idea of annual average departmental expenditure on the programme over the next few years.

²Includes other aspects of solar R & D, not only heating.

³With Agricultural Research Council.

SWEDEN

Politics and power

Wendy Barnaby reports from Sweden on the latest discussion about energy policy

'PRIME Minister Fälldin is thought to be in difficulties', said a Swedish newspaper recently. Considering the energy situation, that is an understatement. Prime Minister Fälldin may well be in his political death-throes.

Only five months ago, after an election campaign in which he promised to rid the country of nuclear power, his appointment as the head of Sweden's three party non-socialist government was widely interpreted as the beginning of the end for nuclear power here. But his government has taken no concrete steps to stop the building—and, in one case, the loading—of reactors. His sympathisers are becoming disenchanted. Over the next few months, the decisions he will have to take will show where his real interest lies: in stopping nuclear power, or in remaining prime minister.

By 31 March the government must advise the Oskarshamn Power Company whether state credit guarantees will be given for the building of the Oskarshamn 3 reactor, now on the drawing board. The decision will also be significant for the owners of the Forsmark 3 reactor, which is at the same stage. The bill, specifying the conditions under which the building of reactors may continue, is expected to be made law in the middle of April. Under its provisions, the owners of any reactor being planned or under construction must present the government with concrete proposals for the 'completely safe' storage of unprocessed fuel or of highly-radioactive waste if the fuel is to be processed: reprocessing agreements must also have been concluded. If these conditions are not met, the government will stop work on the reactor(s) concerned at the end of the year and will compensate owners for costs incurred until the law comes into force in May. Compensation for money spent after May will be subject to special investigation by the government, which has said that it will not pay any bills incurred after it has become obvious that the conditions will not be met. Just exactly when this point is reached will be decided by the government and impartial advisers.

The conditions specified in the bill will have their first test this summer, when the government will have to decide whether or not to allow the loading of Ringhals 3 reactor which is scheduled for 15 July. It will probably

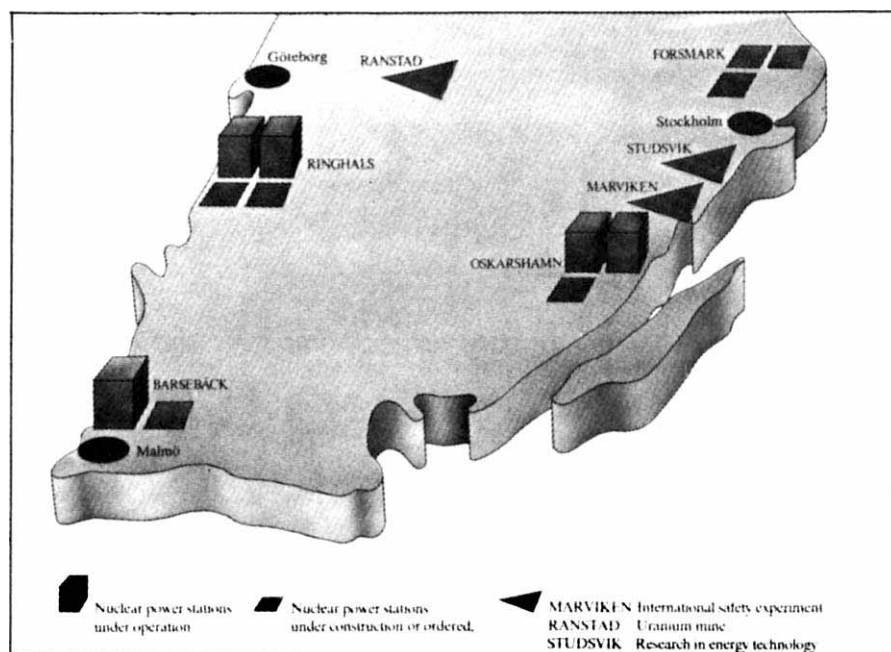
be delayed, however, by a legal case brought by environmentalist Björn Gillberg and his lawyer, Gunnar Michelson, who are appealing to the Supreme Court to overrule a decision made last year allowing the release of $175 \text{ m}^3 \text{ s}^{-1}$ of cooling water from the Ringhals 3 and 4 reactors into the sea. There is at the moment neither a reprocessing agreement nor a storage plan for Ringhals 3. In October, the fate of Barsebäck 2, which is already loaded, must be decided—and the loading of Forsmark 1, due to begin next January, will provide the third test under the new law. The main findings of the government's Energy Commission—which is responsible for formulating an energy policy up to 1990 (including a plan for scrapping nuclear power in the mid 1980s)—are expected to be published next March. In the usual Swedish way they will be commented on by all interested parties before the government presents its proposals to parliament late in 1978.

None of these decisions will be easy. The three coalition parties are nowhere near agreed on energy. The Conservatives want 13 reactors by 1985, the Liberals, 11; and both want to retain the government's freedom of action until the Energy Commission's findings are known. The Centre Party would find it impossible to agree to this without finally damning itself as the party which compromised its anti-nuclear faith when faced with the temptations of power. But whatever decision Fälldin takes will bring him enormous problems. If he decides to stick to his election promises, he will

be faced with unemployment in the nuclear companies and their sub-contractors, estimated to be as high as 40,000 man-years at a time when industrial unemployment is already worryingly high. If he calls a new election, it is by no means certain that he would win: the latest opinion polls show that the popularity of the Social Democratic opposition is rising. Another option would be to have an advisory referendum; but a special parliamentary bill would have to be passed by majority vote. As the Social Democrats are opposed to a referendum, the government parties would have to unite on the bill; and even that co-operation is in doubt.

Fälldin is confident that the nuclear power companies will not be able to meet the conditions of the new law. Jonas Norrby, Director of the State Power Board—which is building more reactors than anyone else—is just as confident that they will. The nuclear companies are cooperating in their attempts to find 'secure' solutions to the disposal problem, and expect to present a position paper on the subject next September. They are predicting that the political instability will finally put their reactors into operation.

The latest idea for disposal involves encapsulating the waste in ceramic material and then compressing it into a solid harder than granite. This suggestion has come from Asea-Atom, a company which is not building any reactors at all, but which is experienced with high pressure techniques in areas other than nuclear waste. It admits that only time and experimentation will tell whether the idea will work. One of those who doubts that it will is Gösta Wranglen, Professor of Cor-



rosion Science at the Royal Institute of Technology. He foresees problems arising from the radioactivity of the waste, which would cause the release of oxygen from the water surrounding it. The resulting oxidising environment would not only favour the spread of plutonium but would also oxidise the pyrites in the bedrock into sulphuric acid, making the water extremely corrosive.

Professor Wranglen is doubtful whether any man-made material could withstand such corrosion for thousands of years, but says that, if the waste must be buried, it should be encapsulated in fire-proof steel with a coating of gold about one-tenth of a millimetre thick: thick enough to ensure absolute impenetrability. To avoid the enormous expense of such a method, he suggests that high-level radioactive waste should not be buried at all, but should be stored under supervision in concrete bunkers or drained rock chambers above the ground water table. The heat from the waste would keep the steel cylinders hot and dry for centuries, and they would need no encapsulation at all. He

argues that resistance to this solution is purely psychological: acceptance of nuclear reactors themselves implies acceptance that the fuel and waste within them might be used in wars or other catastrophes, and we ought therefore to accept a similar risk in connection with stored waste.

In the face of all these uncertainties, it is hardly surprising that gas as an energy source has become attractive. One of Sweden's ship-building companies, Kockums, recently presented a plan in which the need for nuclear power could be reduced from an estimated ten reactors to six by 1985 if liquified natural gas (LNG) were imported from Arab countries. By 1985, gas would provide 26% of the country's energy needs; dependence on oil would stand at 37% instead of the 47% now expected, and nuclear power would provide 11% instead of an expected 29%.

After the announcement of this proposal, a Swedish-American team, which has been doing research into ways of stopping leaks from underground gas storage chambers, declared that a large ship fully loaded with LNG

would have an energy equivalent of a tactical nuclear weapon, and questioned the wisdom of letting such a potential disaster into the Baltic.

Looking further ahead, Sweden may be able to import natural gas from Norway through a pipeline off the projected Norway-Denmark-West Germany line whose parameters are being discussed in Oslo. According to the Norwegians' calculations, gas production would have to be 20 billion cubic metres a year to make the pipeline pay. Present production is only half of this and Norway's Prime Minister, Odvar Nordli, is reported to have refused to guess in which decade the project could begin. All of this can provide only cold comfort for Fäldin: he needs solutions right now. □

● Last week, Mr Alexei Kosygin, the Soviet Premier, opened Finland's first nuclear power station—a 440 MW pressurised water reactor—in Loviisa, a small town south of Helsinki. The order for the power station was placed with the Soviet Union in 1970 at a value of £25 million at the then rate of exchange. □

PAKISTAN

Bones of contention

Azim Kidwai looks at the place of science in the recent elections in Pakistan

AFTER an exceptionally emotional and at times hurtful election campaign, the ruling Pakistan People's Party led by Mr Zulfikar Ali Bhutto swept the polls in the elections held for the National Assembly and the Provincial Assemblies on 7 and 10 March respectively. The main opposition, the Pakistan National Alliance, has challenged the results and has refused to accept them on the grounds that the elections were rigged. It boycotted the provincial elections of 10 March saying that the National Assembly elections had been unfair.

Without going into the polemics of the political scene, and accepting that the People's Party is to govern the country for the next five years, certain projections can be made on the emphasis which will be given to science and technology and the future shape of science policy in Pakistan. The policy kept in abeyance while everyone was preoccupied with the elections is now likely to be finalised, but the lessons learned from the elections might distort it to a certain extent; the first priority, one would expect, would be geared to meeting the practical needs of Pakistan

as reflected during the campaign.

Science policy as such was not an election issue, and was not accorded any status as a separate issue in the manifesto of either of the major parties. The consequences of scientific and technological advance were nevertheless bones of contention. For example, the unprecedented price-spiralling during the past four years—an almost 100% rise—was the chief subject of debate in almost all election meetings. The National Alliance promised to slash prices by good management, by coming down heavily on the black-market and so on, and some of its leaders said they would bring them down to 1970-71 levels. The people's Party refuted the claim, saying that inflation was a complex international phenomenon and that a developing country, weak in basic science and mainly producing raw materials, had to pay through the nose for finished goods.

According to its manifesto, the People's Party is committed to making Pakistan self-sufficient in grain and oil within the next five years, and to a 50% increase in GNP without neglecting industry and defence. With its victory, therefore, agriculture and oil might take precedence; the polls reflected the greater popularity of the People's Party in the rural areas than in the urban centres (it lost heavily in

Karachi, the largest city in Pakistan), probably because it is likely to fund the agricultural sector handsomely.

Agricultural research and inputs like fertilisers, pesticides, new varieties of seeds, and the Irrigation, Drainage and Flood Control Council are likely to be favoured in the development effort. The importance of agriculture in Pakistan is demonstrated by the fact that in 1975-76 it contributed no less than 39,000 million rupees (16 rupees=£1) to a total gross domestic product of 119,000 million rupees. It also happens to be a source of livelihood for almost three quarters of the rural population, which makes up about 70% of the people of Pakistan.

Farm mechanisation could be another priority area. In 1975-76, some 11,000 tractors were imported—a 100% rise over the 1969-70 figure; tillers are to be imported from China and Japan. Productivity in agriculture is still growing very slowly, and is likely to gain impetus through the use of the available scientific and technological techniques.

Luckily for Mr Bhutto, sizeable quantities of oil were found last December at Dhodak in the north. Although the site is primarily a large gas-field, it is estimated to hold about 200 million barrels of oil which can start flowing in the next two or three years. Oil exploration and refineries are foremost on the agenda of the People's Party government. □

IN BRIEF

SRC appointment

It is now widely believed that the next chairman of the Science Research Council will be Professor Geoffrey Allen, who will succeed Sir Sam Edwards in October. Professor Allen, 48, is at present at the Department of Chemical Technology, Imperial College, and was until 1975 Professor of Chemical Physics at Manchester University; his interests are largely in polymer science. He has done considerable committee work for SRC, where there seems to be general satisfaction at the prospect of his appointment.

Delaney Amendment review

The recently announced ban on the artificial sweetener saccharin in the United States and Canada has prompted a major review of an uncompromising provision in the food and drug law which forbids the use in food of any substance found to cause cancer in test animals. Known as the

Delaney Amendment, the clause has long been a target of attacks by the food industry. It has been staunchly defended by many scientists and consumer groups as a prudent measure to keep carcinogens out of the food supply. Within days of the announcement of the proposed ban, a score of bills calling for repeal of the amendment was introduced into Congress, and last week the Secretary of Health, Education and Welfare, Joseph Califano, announced a review of the Amendment's workings.

RANN resignation

The Director of the National Science Foundation's major programme of Research Applied to National Needs (RANN), Alfred J. Eggers, has asked to be relieved of his duties at the end of June to devote more time to studying problems affecting the growth and health of society. Eggers, who has steered the \$70 million RANN pro-

gramme through a minefield of Congressional criticism in the past few years, offered his resignation in a lugubriously worded memorandum suggesting that "in the largest sense of our future activities with our natural resources in our environment, it may well be that in important respects we are entering a 'zero sum game'".

Fast breeder wanted

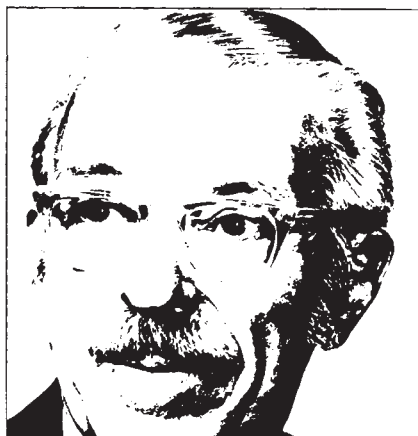
The Highlands and Islands Development Board in Scotland would welcome with open arms the commercial fast breeder reactor (CFR) at Dounreay. At a one-day meeting in Glasgow last week that coincided with the closing-down ceremonies on the Dounreay Fast Reactor (DFR), Professor Kenneth Alexander, who chairs the development board, pointedly observed that the number of employees at Dounreay would rise to 4,500 if the CFR were built there but would sink to 500 if it were not.

A DISPUTE is now raging over permission for landing the Concorde in New York Kennedy Airport. Some of my friends in England and in France tell me that they regard the American prohibition of Concorde landings as an insufferable discrimination. They point to numerous violations of environmental safeguards in the USA, to the concerted din of jetliners taking off in droves from American airports, and to the sonic booms of American military jets. The French say that the American objection to the Concorde is based on the 'PFI' doctrine (*pas fait ici*).

Against these protestations, I point out to them that the supersonic transport (SST) has become a dominant symbol (and a convenient three letter slogan) to American environmentalists; a tangible epitome of technology run wild beyond the bounds of necessity or justifiability. The noise made by Concorde is said to disturb the mating process of wild birds, a ritual that has taken on an almost sacred significance ever since Julian Huxley published his fascinating observations on the sexual foreplay of Great Northern Divers. Environmentalists point to the Concorde as being wasteful of fuel and as being used only by very wealthy people. The needle-like Concorde is said to threaten the delicate ozone layer, which we have come to regard as being like a fragile soap bubble that envelops our atmosphere, protecting us from skin cancer caused by ultraviolet rays from the sun.

In addition to this injurious effect, however, ultraviolet radiation has a

needed role of preventing rickets by its nutritional action. To achieve normal calcium metabolism, most mammals and birds must expose their skins to the sunlight. It is possible to speculate that the spread of the human

Pas de Concorde**THOMAS H. JUKES**

species to northern latitudes occurred because of a mutation that led to the loss of skin pigment. The pale-skinned mutants could utilise sunlight better for production of vitamin D in their skins than could their darker fellow-human beings. Sunlight was scarce and feeble during the long northern winters, but there was just enough for pale complexioned people and so, we speculate, the whites moved into the temperate and subarctic zones. Once there, they eventually found that the

need for direct sunlight by their infants could be alleviated by eating fish liver oils.

But the long era of dependence on sunlight did not really come to an end until a tray of hog millet was pushed under a quartz mercury vapour lamp for ten minutes at the University of Wisconsin by Steenbock and Black. The irradiated millet prevented rickets when added to the diet of rats. In so doing, the millet had served as a 'way station' between ultraviolet radiation and the bodies of animals. From then on, it was no longer necessary to use direct irradiation for preventing rickets in babies. Preformed vitamin D now could be added to food at a trivial cost.

Another evolutionary development, probably a very ancient one, led to the appearance of a biochemical system for combating the carcinogenic effects of ultraviolet radiation: the complex system that removes thymine dimers from DNA, formed by such radiation. The dimers and some adjoining nucleotides in one of the strands are snipped out and removed by enzymes and other enzymes restore the DNA to its original state. Some individuals lack the repair system as a result of a genetic defect, and they are highly susceptible to skin cancer.

We have evolved in concord with our surroundings. Ultraviolet radiation is an example of a stimulus that has a beneficial role at low levels, but is harmful in excess. I am more optimistic for the future of the ozone layer than for the supply of jet fuel.

correspondence

Physical science in Chile

SIR,—My friend and former colleague Alwyn Eades, who recently spent three extremely busy weeks in Santiago, is reported (10 February, page 486) to have said that "it is very easy to be misleading about Chile and very easy to be misled". The truth of this statement is made patently clear by Eades' own article, "Three years after Allende", appearing in the same issue. The article is a mixture of factual information with some personal interpretations and political opinions; it also suffers from some important, and surely involuntary omissions. The unaware reader may find it difficult to distinguish between the first three of these and may not know about the latter; so he may easily be misled by an article which may easily be misleading, especially when the atypical front cover of the issue is politically leading.

Personal interpretations and political opinions are of course entirely subjective and Eades is entitled to make and have his own, but *Nature* is not the proper place to discuss them and I will not do so. I must, however, correct some of his most important omissions and errors lest it be thought that I and/or the Chilean Physical Society share all of his views about what has happened to the physical sciences in Chile.

Eades' account of the development of physical research in Chile is in general accurate. When he writes, however, about the talks for "developing a science policy for Chile" in the early 1970s—which did in fact take place but led to nothing definite—he fails to mention what had been done before and has been done since along these lines. He seems to ignore that the National Commission for Scientific and Technological Development (CONICYT), which was established before Allende in 1968 amongst other things to collaborate in developing such a policy, produced a draft for a National Plan for Scientific and Technological Development in 1974. This draft was carefully studied for several months by a committee in which an important role was played by a sizeable number of leading scientists (including several physicists) who were freely nominated by the universities and by the local Academy of Sciences. The outcome of the committee's work was a much improved version of the plan, which was officially

approved by Chile's highest authorities early in 1976. These important omissions may leave the unaware reader of Eades' article under the wrong impression that the development of a science policy for Chile was completely abandoned after Allende and that no official steps had been taken before him.

When Eades writes about what has happened in the physics department of the Engineering School, where I work, he mentions "several waves of sackings for a mixture of political and economic reasons, as a result of which the number of academic staff has dropped from 45 to about 25"; he is wrong. So far there has been a reduction of only one member of staff (and this for economic reasons) and this affected four members of the department's academic staff: one of them had reached the minimum legal age to retire and had to do so and three of the youngest ones were transferred from full-time to half-time positions. The latter three preferred to resign and found jobs in other local institutions. Independently and at a later time, two other people left this department to work in the physics department in the Faculty of Sciences. Over the past three years, a few others have voluntarily taken posts abroad and some who were on study leave abroad chose not to come back. All this has taken the number of academic staff down to its present value of 32.

It is true that four laboratories in this department lie virtually abandoned; but Eades fails to mention that three of these were never in full operation because of the shortage of staff (one of them was a one-man job) and that the fourth was almost abandoned when the Argentinian physicists who formed it were asked to leave the country before Allende's time. Eades also says about one of the Chilean Physical Society's present preoccupations, that "since all elections are prohibited in Chile, it (the Chilean Physical Society) is unable (like many other organisations) to replace those committee members who leave the country or retire", so he thinks that "it may shortly find itself with no committee at all". Elections are forbidden and this was one of our preoccupations while he was here. We have since found, however, that there are legal ways for scientific bodies such as the Societies of Biology, Chemistry, Physics and Mathematics to renew their committees at their own free will and that

some had in fact already done so. I am glad to inform him and your readers that we have now replaced one committee member who left the country and another who resigned, and that we are planning on renewing our committee early next year, when most of our membership will gather at the University of Concepción for the next scientific meeting.

Finally, I strongly disagree with Eades' statement that one side-effect of the coup was to set back physics research in Chile by nearly two decades. As his article rightly mentions, twenty years ago there was little or no research in physics in Chile: the first groups were just beginning to form, there was virtually no equipment, the Chilean Physical Society did not exist and the very idea of doing physics research in Chile was thought to be utopian by most. Whatever the results have been of the permanent brain drain from Chile over the past twenty years and the departure of scientists with every change of government, we are now much better off indeed than two decades ago. The large (by Chilean standards) number of scientific papers published in the last three years by Chilean physicists who are still working here is the best proof of this.

Yours faithfully,

CLAUDIO GONZALEZ

President,
Sociedad Chilena de Física,
Chile

Not rats, but mice

SIR,—We 'applied biologists' at the Pest Infestation Control Laboratory have been professionally associated with rats and mice for some thirty odd years, in all parts of the globe; I seem to have become our 'coprologer'.

Thomas Jukes (10 February, page 491) has made a slight error: it is not rat faeces that are unscreenable from wheat kernels but mouse faeces. The principle is the same however. I agree with his point about 'filth' in food—it is nearly always purely an aesthetic objection sustainable in the 'developed' world—provided the food has been cooked!

It is a pity therefore that he didn't get that point accurately stated.

Yours faithfully,

R. A. DAVIS

Pest Infestation Control Laboratory,
Surbiton, Surrey, UK

news and views

The inconstant Sun

from David W. Hughes

UNTIL recently astronomers were convinced that the Sun was a regular, well behaved star of considerable constancy. Its present day behaviour was taken to be typical of its activity over a much longer time span. This attitude is now rapidly changing thanks in no small part to the work of John Eddy of the High Altitude Observatory, Boulder, Colorado. He found (*Science* **192**, 1189; 1976) that there was little or no historical evidence to indicate that the 11-yr cycle of solar magnetic and sunspot activity existed before about AD 1700 and concluded (in *Physics of Solar Planetary Environments* (ed. Williams, D. J.) **2**, 958; 1976) that there seems to be a much more gross change occurring in the Sun's behaviour and especially in the solar constant (the energy flux received from the Sun at the Earth's orbit) over periods of hundreds and thousands of years. These changes do not seem to be periodic.

Maunder minimum

The inconstancy of the sunspot cycle was first noticed by F. W. G. Spörer and E. W. Maunder in the 1890s. Spörer was studying the distribution of sunspots with latitude and found evidence that the number of spots in the northern and southern hemispheres of the Sun was not always balanced. To check this he consulted historical records of solar activity and found to his amazement that over a 70-yr period between 1645 and 1715 practically no sunspots were seen. This period has been named the Maunder minimum. The few spots that did occur were invariably at low solar latitudes and were single; only one group was seen between 1645 and 1705. Between 1672 and 1704 there were no northern hemisphere spots at all, in fact there were fewer spots between 1645 and 1715 than in a single active year under normal conditions. Eddy re-examined the historical data (*Science op. cit.*) bearing in mind that 'absence of evidence is not evidence of absence'. He also posed such questions as 'how good

were the 17th century observers and their observing techniques' and 'how constant a watch was kept?'.

Most solar telescopes of those days had focal lengths of 2 to 4 m and apertures of 5 to 10 cm, the solar image being projected onto a white screen placed behind the eyepiece. The solar image was large enough to make spot umbrae and penumbrae easily discernable and observers recorded details of white-light faculae, penumbrae filaments, satellite sunspots and most of the observational details known of sunspots today. Several transits of Venus and Mercury were observed during the 17th century as well as some 53 solar eclipses. The fact that the first division in Saturn's rings was discovered in 1675 and that five of its satellites were discovered between 1655 and 1684 attest to the fact that a resolution of

1 arc s could be obtained and that 11th magnitude satellites less than 40 arc s from the limb of a bright planet could be observed. Eddy concluded that spots would have been seen if they were there, a conclusion supported by the fact that the few spots that did occur motivated the discoverers to report their observations immediately in the journals of the time.

Moving from sunspots to phenomena caused by and modified by solar activity Eddy verified Clerke's observation that 1645 to 1715 was a period characterised by a marked absence of aurorae and was also a time of profound magnetic calm. The number of aurorae reported increased dramatically after 1716. Even though the scientific renaissance came to auroral latitudes at that time and Halley's paper in 1716 put the auroral

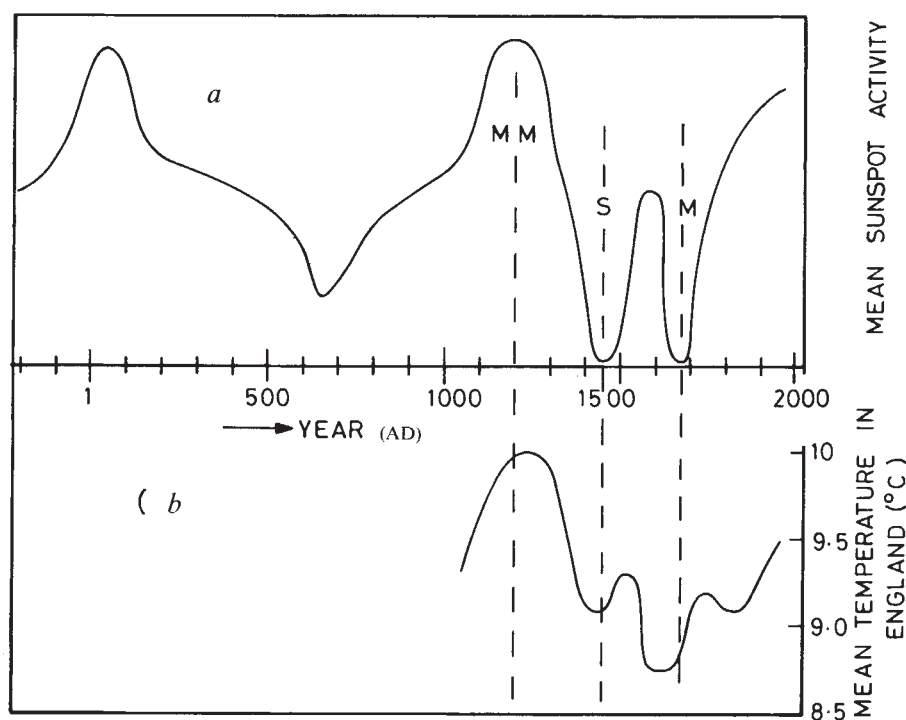


Fig. 1 *a*, A long term envelope over the solar sunspot activity using ^{14}C activity as a guide. MM is the Mediaeval maximum, S the Spörer minimum and M the Maunder minimum (after Eddy in *Physics of Solar Planetary Environments* (ed. Williams, D. J.) **2**, 958; 1976). *b*, Estimate of the mean annual temperature in England (after Lamb, *Climate, Present and Past and Future* **1**, Methuen, 1972).

phenomenon on a firm scientific footing, both of these resulting in an upsurge of interest, the 'Maunder minimum' of sunspot activity is evident from auroral activity alone.

In addition it cannot just be a curious coincidence that the chromosphere was discovered in 1706 and that the first description of a structured corona was given in 1715. Coronal shape varies with solar activity. With many underlying spots the corona is large and made up of numerous long tapering streamers extending outwards like flower petals. At solar minimum, with very few sunspots, the corona becomes compressed and bland, the only unusual features being the long symmetrical extensions along the solar equator, this being the zodiacal light (or F corona). Between 1645 and 1715 the corona at eclipses was always described as being limited in extent (1 to 3 arc min above the limb) and as being dull or mournful, and reddish, typical of an extended minimum of solar activity.

Radiocarbon data

Now sunspot data only takes us back with any reliability to the early 17th century. Radiocarbon data on the other hand can extend our knowledge of solar activity back to about 5000 BC, almost half way to the last glaciation. ^{14}C is produced in the Earth's upper atmosphere by galactic cosmic ray bombardment. Through the intermediary of the interplanetary magnetic field the Earth can be shielded from these galactic cosmic rays, high solar activity increasing the field, reducing the cosmic ray flux and lowering the ^{14}C production rate. Trees assimilate ^{14}C as CO_2 in the photosynthesis process so individual tree rings preserve a record of the atmospheric $^{14}\text{C}/^{12}\text{C}$ ratio at the time of formation. Figure 1a shows the overall envelope of sunspot activity obtained when the ^{14}C concentration is used as an indicator. The Maunder minimum can be clearly seen as can the Spörer minimum (1400–1510) and the mediaeval maximum of activity which occurred between 1120 and 1280. At the present we are half way up an increasing branch of the curve moving towards a time of high solar activity. Figure 1 also shows how these massive solar changes affect terrestrial climate. The lower curve (Fig. 1b) shows mean annual temperature in England and it can be seen how the Maunder and Spörer minima correspond to the two coldest extremes of the Little Ice Age when global temperatures were depressed by 0.5°C to 1°C .

Solar variability

Eddy is convinced that the individual ups and downs of the 11-yr cycle and

also shorter term solar variability are almost wholly unrelated to terrestrial meteorology, this in fact being governed by the total radiative output from the Sun (which is proportional to the solar constant). Öpik (*Irish Astr. J.* **8**, 153; 1968) found that the average value of the measured solar constant had increased steadily, by about 0.28% during the 30-yr period between 1921 and 1952. Figure 1a thus also represents the 1% peak-to-peak amplitude variation of the solar constant. Sunspot production is thought to be directly proportional to the rate of radiation flow through the solar photosphere.

In a recent paper in *Solar Physics* (**46**, 3; 1976) Eddy, Gilman and Trotter found that the equatorial rotation rate of the solar photosphere just before the Maunder minimum was 3 to 4% higher than its present value. The rate of change of rotation rate between 0 and $\pm 20^\circ$ solar latitude was also enhanced by a factor of 3. These results came from an analysis of a series of daily full-disk drawings of the Sun made by Johannes Hevelius between 1642 and 1644 and published in his book *Selenographia Sive Lunae Descriptio*. This anomalous rotation may be just beyond the velocity range capable of producing sunspots by the solar dynamo action so the high velocity in the early 1640s could have caused the sunspot dearth that followed.

Dynamo theories of solar magnetic activity rely on the stretching of the general magnetic field of the Sun by a radial gradient of angular velocity in the subsurface convection zone. To explain the migration of sunspots towards the solar equator as the solar cycle progresses this angular velocity must increase with depth below the photosphere. Redistribution of angular momentum throughout the convection zone caused by a variation in the radiation flux and resulting in a decrease in angular velocity deep in the zone could reduce the strength of the dynamo action and switch off the spots.

Tidal influence unlikely

It has often been suggested that the 11, 78 and 179-yr cycles of solar activity are controlled by gravitational tides raised on the Sun by the planets, even though this tidal force is miniscule compared with the force of solar gravity (which is about 10^{11} times larger). Proponents of the tidal hypothesis tend to consider the tides as triggers but as Charles Smythe and John Eddy point out on page 434 of this issue of *Nature* trigger mechanisms have the disadvantage of being out of the reach of physical criticism

and usually are beyond the grasp of observational tests. Tidal theories also produce regular sunspot periodicities so how can the Maunder minimum be produced? Planets surely did not stop exerting tidal influence when the spots disappeared.

Using a computer Smythe and Eddy calculate all the two, three and four planet conjunctions that occur between AD 1575 and 2000 and compare their occurrence times with solar activity cycles. There are no dissimilarities between the conjunction frequencies that occur within the Maunder minimum period (1645–1715) and the ones which occur at adjacent periods. They go on to calculate the daily tidal potential at the solar equator exerted by the planets. The power spectrum of this potential, over a 50-yr period, has principal peaks at J , $J/2$ and $J/3$ (J being the Jovian orbital period of 11.86 yr). The authors find that the power in each of these peaks has not changed perceptibly in the past 500 yr, the power spectra for 1925–1975 being indistinguishable from the Maunder minimum spectra. Tidal theories of sunspot formation thus become much more difficult to support.

So we are presented with an inconstant Sun with sunspots which can be switched on and off in about 10 yr and can stay off for around 70 to 160 yr. The spottiness seems to be tied directly to the total energy flux from the Sun and this seems to vary with time by about 1%. Why it varies and how it directly affects spot production are two questions still to be answered. □

H-2 and mating preferences

from J.C. Howard

It ill becomes an immunologist to draw attention to the work I want briefly to discuss. Unhappily, the paper appeared in a journal specialising at the moment in the advancing front of immunology, and was probably rather little read by most of the people best qualified to enjoy it. The paper, entitled 'Control of mating preferences in mice by genes of the major histocompatibility complex' (Yamazaki *et al. J. exp. Med.* **144**, 1324; 1976) reported that, given a choice, a male mouse will tend to pick a female mouse to impregnate on the basis of the alleles she carries in her major histocompatibility (H-2) system. Furthermore the preference shown by the male is itself a function of his H-2 alleles. The preference is not necessarily for the self H-2 type; in some matings it is, for instance BALB.B(H-2^b) δ chose H-2^b ϕ

in 70% of trials, in preference to BALB (H-2^b) ϕ , but in another cross B6 δ (H-2^b) showed a preference for B6-H-2^k ϕ , and B6-H-2^k δ for B6 ϕ , rather than for the partner of their own H-2 type. The experimenters used H-2 congenic mice to avoid confusing influences from the genetic background.

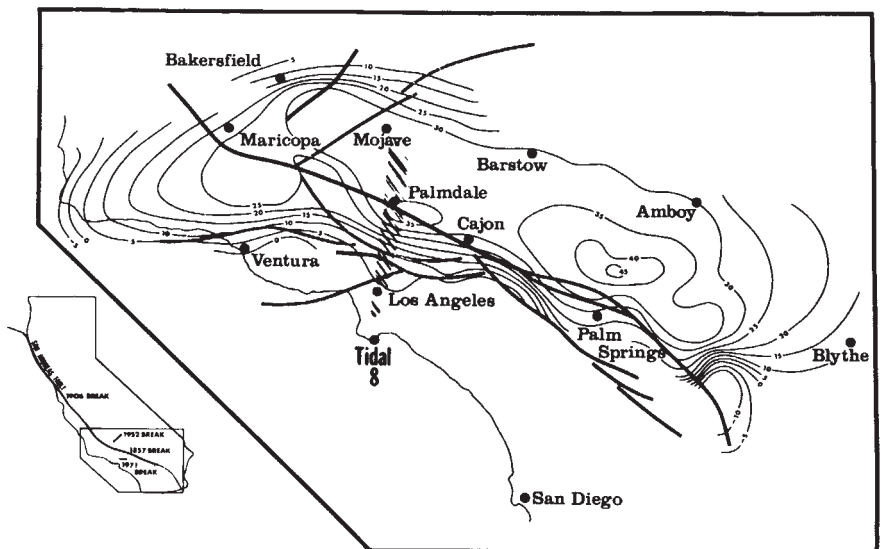
This very strange and remarkable phenomenon was appropriately discovered at the Sloan-Kettering Memorial Cancer Center since it was the Sloan-Kettering's President, Lewis Thomas, who first suggested at the second International Congress of Immunology in Brighton in 1974, that the diversity of histocompatibility antigens might be used by animals to identify each other, presumably by smell, thus drawing together two of his persistent interests, immunology and pheromones.

There are many aspects of these findings that need further clarification: do the females do any choosing, or do they merely wait to be chosen? Is the stimulus indeed a smell, and if so from where (the urine?), and precisely what is smelly? Histocompatibility antigens are not obvious candidates for airborne pheromones: perhaps the association with H-2 is indirect, through metabolites from characteristic bacterial flora infesting the skin, or intestinal and genitourinary tracts, the presence of particular organisms being under the control of H-2 linked Ir genes. In this context it was reported a few years ago that the bacterial flora of the human skin was indeed individual, as assessed by characteristic patterns of phage sensitivity, and of course the individuality of human odour has long been known, at least to dogs.

The biological significance of these complex phenomena is not clear. As Thomas and others have pointed out, the recognition of individuals is important for animals with a developed social structure. The H-2 associated mating preferences demonstrated by Lewis Thomas and his colleagues have two additional interesting consequences. In so far as the mating preferences are disassortative they will encourage the persistence of H-2 polymorphism. For a long time, evolutionarily inclined immunologists (and indeed immunologically inclined evolutionists such as J. B. S. Haldane) have been looking for possible 'external' reasons why H-2 polymorphism should be so extensive and so persistent. Until very recently there were no good clues. For a time the effect of H-2 incompatibility on placental weight in mice was offered as a mechanism for the maintenance of the polymorphism: foetuses H-2 incompatible with their mothers had larger placentas and a heavier birth weight than H-2 compatible foetuses in the same pregnancy. There were difficulties in repeating the work, and it has now dropped out of fashion.

Palmdale bulge changes shape

from Peter J. Smith



It is just about a year since Castle *et al.* (*Science* **192**, 251; 1976) reported their discovery of the crustal bulge centred near the town of Palmdale about 65 km north of Los Angeles. This uplift, which apparently began about 1960, had by 1974 reached more than 0.35 m at its highest point and was observable over an area of at least 12,000 km² of southern California. Interest in the bulge was enhanced by the fact that Palmdale lies almost on the San Andreas fault and by the discovery that the uplift contours were elongated along the fault over a distance of more than 150 km. Moreover, most of the area with an uplift greater than 0.15 m had remained virtually aseismic since at least 1932. The possibility that the bulge might herald a major earthquake therefore had to be taken seriously.

No such earthquake has yet occurred, but a new analysis of historical and recently acquired geodetic data, announced this month by the US Geological Survey, reveals that the uplift covers a much wider area than previously supposed and that part of it has collapsed since 1974. The zone originally studied by Castle and his colleagues lies between Maricopa and Cajon (see map); but it is now evident that uplift extends at least as far as Blythe in the south-east. The bulge thus stretches for at

least 600 km along the San Andreas fault and covers an area of more than 90,000 km². Moreover, there is a second peak of uplift (between Palm Springs and Amboy) which is actually greater than the Palmdale peak in 1974.

Since 1974, however, the uplift in the vicinity of Palmdale itself has been reduced by about 0.18 m. The significance of this depression is unclear, although the area has seen similar behaviour before. A less well documented uplift of possibly as much as 0.30 m took place sometime between 1897 and 1914 but had almost disappeared by 1926, which has led Castle to speculate that "the recent deformation may be but a part of a generally cyclic process".

It may be significant that no major earthquake can be associated with the earlier episode of uplift and collapse, which would seem to imply that the present deformation does not necessarily indicate an imminent earthquake. On the other hand, uplift definitely preceded the 1961 Niigata (Japan), the 1971 San Fernando (California) and the 1975 Haicheng (China) earthquakes, among others. The 'Palmdale bulge' may or may not pose a problem to the residents of southern California, therefore. The only thing to do for the time being is to continue to observe it as closely as possible.

Furthermore its universal significance as a mechanism for maintaining polymorphism seemed doubtful when it was realised that it was hardly applicable to the birds!

More recently Doherty and Zinkernagel provided a simple mechanism for

heterozygous advantage in H-2 when they showed that viral antigens on cell surfaces were recognised in association with H-2 specificities. The virus-infected H-2 heterozygote is thus able to display a more varied antigenic stimulus to itself than the homozygote, with a

correspondingly larger protective immune response. This is the kind of effect that not only guarantees the persistence of H-2 polymorphism (though not necessarily its variety) but also may explain why the mouse, at least, goes to such lengths to increase H-2 heterozygotes in the population above the 'natural' equilibrium frequency, by using recombination inhibition from the neighbouring T system in combination with a high frequency of lethal recessive alleles. Disassortative mating preference by external identification of H-2 products or their effects will operate in the same direction.

Recognition of genetically endowed polymorphic pheromones may also play a part in maintaining the genetic integrity of incipient species in the face of a constant risk of interbreeding with the parent species. John Godfrey has shown for example, that subspecific variants of the bank vole *Clethrionomys glareolus* show a pheromone-dependent assortative mating preference for their own subspecies, given a choice. Some behavioural isolation is clearly a prerequisite for fixation of the subspecific gene complex: it would be interesting if the accumulation in a subspecies of polymorphic variants at genetic systems normally recognised as antigens were to play a part in this important phenomenon.

Clearly the Sloan-Kettering findings cannot be taken lightly. It is therefore a pity that the H-2 associated mating preferences shown by the use of congenic lines were not confirmed in breeding tests, since trivial and unrecognised differences between lines, for example in housing, handling and maintenance could possibly have caused the effects that were seen. I hope that the work is soon confirmed and extended, and that the study of major histocompatibility polymorphisms has just begun a new life far from the inward mysteries of the immune response. □

Landslides in sensitive soils

from I. J. Smalley

THE property of many sensitive clay soils in eastern Canada to transform suddenly and unexpectedly to material of virtually zero strength makes this region particularly prone to large landslides. Since the turn of the century, landslides in eastern Canada have cost more than 100 lives and involved the loss of more than 20,000 acres of upland. Another large landslide may threaten this spring on the calculation that these massive earthflows occur on average every four or five years and the last one, in which 31 people were killed, occurred at St Jean Vianney in 1971. 'Sensitive' soils have

a very high ratio of undisturbed strength to remoulded strength but the nature of the transition and the factors which control it are not yet established despite the investigations which have followed in the wake of the St Jean Vianney disaster.

The Terrain Sciences Division of the Geological Survey of Canada has carried out extensive mapping in sensitive soil regions, and the geological setting of the problem in eastern Canada is becoming clearer (Gadd, *Proc. 4th Guelph Geomorphology Symp.* 137; 1975). Extensive studies of the pore water chemistry (Torrance *Can. Geotech. J.* 12, 326; 1975) suggest that the leaching effect first described by I. T. Rosenqvist in the late 1940s should still be considered a significant factor in Canadian clays, although there appears to be no direct correlation between leaching and sensitivity. Another interesting lack of correlation is that between sensitivity and size of landslide; a conclusion reached by Mitchell and Markell after a study of 75 landslides (*Can. Geotech. J.* 11, 11; 1974).

Interparticle cementation, by producing strong but brittle bonds, could be an important factor contributing to sensitivity. Attention has been focused recently by R. N. Yong and his collaborators at McGill University on the presence and importance of amorphous matter as a cementing agent in these clays and it seems that some soils may have an amorphous matter content as high as 40%. Mineralogical studies on sensitive soils still present problems, mainly those of making quantitative estimates of constituents. Some initial thermogravimetric studies have been made on clay from the St Jean Vianney slide and further studies are in progress on material from the vicinity of another large 1971 event—that at South Nation River. The lack of clay minerals is notable; Gillott (*Can. Mineral.* 10, 797; 1971) was one of the first to draw attention to the predominance of primary mineral particles (as distinct from clay mineral particles) in the Canadian sensitive soils. The soils, as a result, have a very low plasticity index and the low plasticity facilitates the solid-liquid transition. Cementation provides extra strength but once the interparticle bonds are broken there are insufficient clay minerals to influence the abrupt change in strength properties.

Mitchell and Klugman (*Engng Geol.* in the press) in a survey of recent Canadian work report that regional differences in sensitive soils are becoming more apparent although the occurrence of landslides of the characteristic form may be more widespread than has been realised. Critical

factors may be revealed when the scope of investigations can be enlarged to include the less accessible regions, in particular Labrador. An improved classification of sensitive soils is also required and R. Söderblom at the Swedish Geotechnical Institute has recently taken a significant step in this direction (*SGI Prelim. Rept* 55, 1974). He is concerned with soils of extreme sensitivity (often called quick-clays, after the Swedish term *kvicklera*) and has distinguished two distinct types. Those which require a considerable energy input to cause the solid-liquid transition he calls 'slow quick clays' and those which transform easily are 'rapid quick clays'. The new parameter of rapidity can be assessed using a standard Casagrande liquid limit device, and should prove very useful when sensitive soil comparisons are being made. □

Relapses in malaria

from F. E. G. Cox

RELAPSES, months or even years after an initial infection, are characteristic of malaria and in the quartan disease an individual may experience periodic fevers for the whole of his life. Until the discovery of the exoerythrocytic stages of malaria in the liver in 1948 it was widely assumed that during the remissions the parasites persisted in the blood at subpatent levels and that their spasmodic appearances in larger numbers represented recrudescences rather than true relapses. Relapses proper only occur when the blood is quite free from parasites during the intervals between periods of patency. The discovery of exoerythrocytic stages in the liver immediately suggested that it was there that they sojourned and from time to time flooded the blood with new erythrocytic forms. A number of observations, however, have indicated that this explanation is too simple (Garnham *Protozool. Abstr.* 1, 1; 1977) and recent work in the Soviet Union and Romania suggests an alternative hypothesis.

It has been suspected for some time that the tertian malaria parasite, *Plasmodium vivax*, exists in two forms, one with a short prepatent period (13–16 d) before parasites appear in the blood following infection from a mosquito and the other with a less clearly defined longer period of 300 d or more (Sergiev & Tiburskaya *Arch. Roumaines Path. exp. Microbiol.* 26, 475; 1967). The short prepatent form is characteristic of tropical strains and the other is typically found in more temperate regions such as the Soviet Union and North Korea. Using a North Korean strain a team of nine coworkers

ers, six British and three Romanian, (Shute *et al. Trans. R. Soc. trop. Med. Hyg.* **70**, 474; 1976) have demonstrated the coexistence of sporozoites capable of inducing long or short prepatent periods. They infected humans with 10 , 1×10^2 , 1×10^3 or 1×10^5 sporozoites and found that in those given 10 or 1×10^2 the prepatent periods ranged from 262–628 d, in those given 1×10^3 the prepatent period in one was 16 d and in four others between 257–386 d and in seven infected with 1×10^5 the prepatent periods were 13, 15 or 16 d. In parallel experiments using a tropical strain of *P. vivax* all the prepatent periods were short, 12–17 d (Ungureanu *et al. Trans. R. Soc. trop. Med. Hyg.* **70**, 482; 1976). These experiments suggest that temperate strains of *P. vivax* consist of at least two genetic types, one with a long and one with a short prepatent period and that in any population of sporozoites there are more long prepatent period forms than short whereas in tropical strains the reverse is probably true. The selection of the two forms has presumably been due to differences in the seasonal availability of suitable mosquitoes for transmission.

It may well be that there are more than two forms of *P. vivax* within any one strain and Tiburskaya and Dukhanina (*Med. Parazit.* **45**, 218; 1976) believe that there may be as many as five in one Russian strain and

that the expression of a particular form varies from month to month.

The life cycle of the malaria parasites must now be redescribed. Sporozoites are injected into the host and enter the liver where they may develop rapidly or slowly into multinucleate exoerythrocytic schizonts the products of which invade blood cells. There is no need to postulate a continuous series of exoerythrocytic cycles to account for relapses because these could result from initial infections with sporozoites having prepatent periods of up to several years. Differences in the time taken for different sporozoites to mature into exoerythrocytic stages could account for the irregular relapses characteristic of malaria.

Continuous exoerythrocytic cycles are now no longer thought to occur in any species of human malaria. In *P. vivax* and *P. ovale*, sporozoites with long but variable development are responsible for relapses whereas in *P. falciparum* and *P. malariae* small numbers of parasites persist in the blood after the initial infection and subsequent attacks of malaria result from recrudescences of these and are not true relapses. It is now, more than ever before, important to know exactly what species is causing malaria because parasites that occur only in the blood respond to a number of antimalarial drugs whereas those that develop slowly somewhere else in the body do not. □

Telecommunications in the '80s

from John R. Forrest

A meeting entitled Telecommunications in the 1980s and After was held at the Royal Society on 10–11 March, 1977. A small exhibition also provided a demonstration of telecommunications hardware currently used or under development.

EXOTIC technology, system planning and social implications were all discussed in a balanced programme. Sir Edward Fennessy (British Post Office) saw an optimistic future for telecommunications on the global scale, with some 1,500 million telephones by the year 2000, this figure being dominated by the now-developing countries. He saw electronic letter and document overnight transmission as a very rich field for development in balancing the loading of telecommunications traffic. This optimism in regard to the almost limitless capabilities of technology was shared by K. Corfield (TEMA Ltd), but he showed concern that Britain should itself have a most modern and internationally-compatible telecommunications system as an incentive for a powerful export industry. By contrast,

IN the heady days when 'polywater' was thought to exist and even to represent a danger to life, theoretical chemists were quick to calculate the stability of water dimers and to risk their reputations by lending weight to credence in the new form of water. The bursting of the polywater bubble has not, however, ended interest in the dimeric species (H_2O)₂. It was first postulated to exist in order to explain pressure, volume and temperature observations in water vapour and later observed directly in solid rare gas matrices and by mass spectrometric techniques. The dimer, trimer and other small polymers are of significance in the understanding of liquid water itself and in the study of the all-important hydrogen bond which must be involved in the stability of water polymers and indeed in most natural polymers.

The quality and reliability of the most recent theoretical predictions using *ab initio* molecular orbital methods is demonstrated by a new detailed experimental study of the water dimer by molecular beam electrical resonance spectroscopy (Dyke *et al. J. chem. Phys.* **66**, 498; 1977). Molecular beams of the

Dimeric water molecules

from Graham Richards

hydrogen-bonded dimers were generated by expanding water vapour through a pinhole nozzle. The cooling so produced reduces the temperature of the beam so that the thermal energy is insufficient to break the relatively weak hydrogen bond. Radiofrequency and microwave transitions were observed arising from changes in the rotational energy of the rotating dimer. This gives structural information as does the dipole moment which can be interpreted to give the relative orientations of the two H_2O monomer subunits.

The resultant structure is the so-called "trans-linear" complex. This consists of one water molecule joined to the other by means of a linear hydrogen bond. The covalently-bonded hydrogen of one partner points along a lone pair electron lobe of its mate with a plane of symmetry along the hydrogen bond bisecting the HOH angle of the proton acceptor. The non-hydrogen bonded protons are disposed *trans*

to the long bond.

The distance between the two oxygens is longer in the dimer than it is in liquid water, a similar result also being known for (HF)₂. Apparently then, when a hydrogen bond is formed, charge transfer occurs in such a way as to enhance the formation of further hydrogen bonds which are strengthened, as is the case in liquid water.

Another striking point is that the geometry of the proton acceptor is nearly tetrahedral in conformity with simple ideas about the disposition of electron lone pairs, despite the fact that calculated electron density maps do not show prominent lone pairs on monomeric water. The results do, on the other hand, support the accepted view that hydrogen bonds have an important covalent component and are not merely electrostatic.

The detailed results of this work answer most of the questions about the water dimer, but many remain about the trimer. Is it an open structure with two hydrogen bonds, or is it a cyclic entity with three? No doubt theoreticians will now feel confident to answer this question long before the difficult experiments can be completed.

Mr. D. Elias (West German Ministry of Telecommunications) was more conservative towards exotic technology, with the exception of techniques like Viewdata, seeing as first priority the full development of conventional telephone service to all members of the public. He also stressed the need to take future system development decisions with careful regard to their effects on employment.

Speaking about integrated circuit technology, D. Roberts (Plessey Ltd) showed that although 1 Mbit of memory on a $1'' \times \frac{1}{2}''$ chip was likely very soon, the progress to more and more logic elements on a semiconductor chip was likely to be checked by the low yields and higher failure rates as complexity increased, implying an optimum scale of integration. He pointed to the crucial need for very much greater contact between telecommunications equipment and semiconductor device manufacturers so that highly-structured logic devices such as telecommunications-dedicated microprocessors could be developed.

For telephone exchanges, digital switching and computer supervision through stored program control with its ease of modification to suit continued system development, was seen as essential by D. Leakey (GEC Ltd) and R. Ketchledge (Bell Laboratories, USA). Savings in cost and equipment size would also result, but advances in software were still much needed. Because of the diversity in population settlement size, hybrid systems involving local microprocessor control coupled to centralised computers were felt to be the optimum solution by K. Cattermole (University of Essex). Since optical techniques were likely to be used in transmission, he also saw optical switching as a very fruitful research area for future wide-band systems.

In transmission techniques, the most exciting development was undoubtedly optical fibre communication. Fibre attenuation was now down to a few dB km⁻¹ in large volume production. Continued research was needed in fibre jointing, fibre reproducibility, laser characteristics and modulation bandwidth, but no fundamental barriers to progress were seen. The optical link for telephone traffic from Martlesham to Ipswich in the UK would eventually be upgraded to a modulation rate of 560 Mbit s⁻¹ from the initial 8 Mbit s⁻¹, providing a full test of possible future systems. Although multi-mode, graded-index fibres had bandwidths up to 1 GHz, A. Gambling (Southampton University) was optimistic over the future for the higher bandwidth single-mode fibres.

The continued growth of submarine

cable installation at around 16% each year was predicted by J. Tilley (STC Ltd). This was one area where it was felt that digital transmission would not replace analogue transmission for some time, a further generation of analogue submarine cables with bandwidths up to 150 MHz being likely. The optical fibre submarine cable was, however, suggested as an exciting possibility for the next century.

There was very similar traffic growth rate on mature satellite links, but here transmission would be overwhelmingly digital in future. The long-term development, in the opinion of B. Edelson (COMSAT, USA), was the construction in space, using the space shuttle, of large satellite platforms containing replaceable equipment modules. Antenna beams would be electronically shaped and steered.

The lectures on planning for the future were almost entirely concerned with the social effects of a highly-developed telecommunication system, which can on one hand integrate nations and populations, or alternatively insulate them from personal

contact. G. B. Thompson (Bell-Northern, Canada), in a highly entertaining lecture, felt that neither extreme would occur, but in his opinion our society did not know how to handle information and ran into trouble when too large a proportion of the population was involved in information processing as opposed to materials processing. The basic difference with information is that you can sell it many times over and you still have it, he commented. He felt that some very careful research was needed before our society could adjust to a highly-developed information transmission system.

The overall feeling of the meeting was that the novel technology required for future telecommunications systems posed little problem, the research and development areas being clearly defined. The real difficulties were in the financial resources required for systems of ever-increasing size and the difficulty of organisation and decision. In the words of R. Ketchledge: "... only politics and lack of vision stand in the way of the new era". □



A hundred years ago

IN an article which lately appeared in *NATURE* (vol. xv., p. 308), I gave an account of certain very remarkable discoveries made by Prof. Kühne, of Heidelberg, which added additional interest to the startling announcement contained in a recent communication made by Prof. Boll, of Rome, to the Berlin Academy, to wit, that the external layer of the retina is, during life, of a purple colour, which disappears at death, but which is, during life, continually being bleached by the action of light.

It is with great surprise that I have heard that the prominence given to Prof. Kühne's researches on the "Vision-purple" in my article in *NATURE* has given some pain to Prof. Boll, who probably feels some disappointment in not having been allowed to remain in sole possession of the promising field of research upon which he had entered.

In the particular case in point it would appear that Prof. Boll re-discovered (and, what is more, *appreciated the full value of*) a fact which had really been observed by some others (Leydig in 1857, and Max Schultze in 1866), but which had certainly not become part and parcel of the common stock of scientific knowledge, viz., that the rods of the retina are red, he observed that under the circumstances of his own experiments the colour faded at death, and arrived at the false conclusion that the colour was a function of the vital condition of the retina. He, however, observed the remarkable

action of light in modifying the colour. "During life", he announced in his paper, "the peculiar colour of the retina is continually being destroyed by the light which penetrates the eye. Diffuse daylight causes the purple tint of the retina to pale. The more prolonged, dazzling action of the direct rays of the sun entirely destroys the colour of the retina. In darkness the intense purple colour is again restored. This objective alteration of the peripheral structures of the retina brought about by the rays of light undoubtedly occurs in the act of vision". This was the great discovery which Boll made, and with which his name will ever remain honourably connected. Although, however, he had been in full possession of the facts in the month of June of last year, when he demonstrated them to Professors Du Bois-Reymond and Helmholtz, and only published his paper in November, he did not succeed in making the discoveries with which, justly, the name of Kühne is now associated. Kühne showed that if the retinal purple is usually destroyed at death, the result is attributable to the action of light, persistence of the colour being by no means necessarily connected with the living condition of the retina. In his beautiful and far-seeing discovery of the true function of the retinal epithelium cells as restorers of the vision purple, he was fortunate enough to make a discovery which it would be very bold for any one—even for Prof. Boll—to say he would have made, had time and opportunities been granted. In saying that Boll had discovered everything referring to the vision purple, M. Warlemont shows that he has not appreciated the fact that two great discoveries have been made, the second supplementing the first, and actually needed in order that the significance of the first should be appreciated.

From *Nature* 15, 29 March, 477; 1877.

articles

Temperature and duration of the shadow of a recently-arrived lunar boulder

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Natural thermoluminescence retained by soil samples from the shadows of some Apollo 17 boulders and from an adjacent sunlit area has been used to derive the temperature in the shade and the length of time for which the shadow has been cast in one case. This has implications for soil mechanics on the lunar surface.

In the near-perfect lunar vacuum, the absence of gaseous convection, and the highly insulating nature of the powdery surface soil, ensure that there are steep temperature gradients across the boundary of a lunar shadow. It is therefore of interest, for example in studying the condensation of volatiles and even of any pristine water vapours, to try to determine the average temperature in the shade on the lunar surface. We have used the thermoluminescence (TL) properties of soil samples from inside the shadows of some Apollo 17 boulders and from an adjacent sunlit area to make such calculations. Some evidence, based on the freshness of boulder tracks observed by the astronauts, suggested that the boulders had not been in their present position for very long. We have therefore attempted to calculate the length of time for which one of the boulders had cast a shadow on our soil sample.

To preserve the natural TL of the shaded samples as much as possible it is important that they should be kept at low temperature after retrieval¹. Some of the samples used in the present study were kept refrigerated by the staff of the Lunar Receiving Laboratory, at $\sim -20^\circ\text{C}$, starting about 3 weeks after the Apollo 17 splashdown on 17 December 1972. Since their receipt by us in England (on 22 March 1974), these samples have been kept in our laboratory at the temperature of either dry ice (-78.5°C) or liquid nitrogen (-196°C). This continuous refrigeration has proved beneficial. For comparison, a counterpart of each of the shadowed samples has been kept at room temperature from the beginning: the 'freezer counterpart samples'. The sample from an adjacent sunlit area has also, throughout, been stored at room temperature.

The samples concerned were collected from two locations at the Apollo 17 site. From Station 6 came the 'permanently shadowed' soil sample 76240, collected from about 1 m under the north overhang of boulder no. 4, which measured $\sim 5 \times 4 \times 3$ m. The boulder is believed to have been derived from the North Massif and seems to have been moving downhill on an 11° slope to the south. For comparison, a second sample (76260, the 'sunlit sample'), consisting of a 2 cm deep skim soil, was collected from just outside the shadow. From the second location, Station 2, only one sample (72320) was collected: a surface soil from about 20 cm under an east-west overhang of a 2-m boulder (no. 2) lying in a strewn boulder

field at the lower slopes of South Massif. This sample seems, from our investigations, to have been only partially shaded. No sunlit sample from an adjacent area was available in this case. Details of the apparatus used by us for TL measurements may be found in ref. 2.

TL glow from shaded and sunlit samples

Figure 1 shows the striking difference observed in the natural TL glow emitted by the shaded and the sunlit samples. The shaded sample from ~ 1 m inside the shadow (76240, 22) has by far the most natural glow, followed by the one from ~ 20 cm inside the shade (72320, 4), with the least preserved glow being in the sunlit sample (76261, 25). Moreover, the glow starts at successively and appreciably higher temperatures on going from the shaded to the unshaded samples in the above sequence (namely, $\sim 55^\circ$, $\sim 110^\circ$ and $\sim 180^\circ\text{C}$, respectively), resulting from thermal drainage of TL from the shallower traps under quite distinct lunar temperature environments.

To investigate the effect of room-temperature storage of the

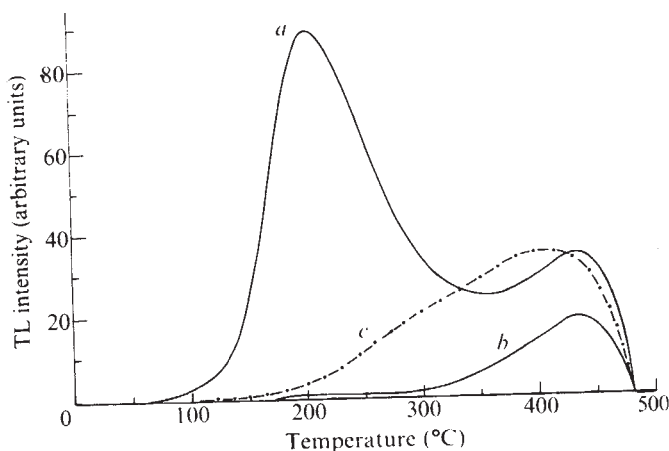


Fig. 1 Natural TL glow curves from the shaded and the unshaded samples. The 'permanently shadowed' sample (76240, 22, curve *a*) and the 'partially shadowed' sample (72320, 4, curve *c*) have been kept refrigerated (see legend to Fig. 2), while the sunlit sample (76261, 25, curve *b*) has been stored at room temperature since receipt on the Earth. Sample weights: *a*, 1.6 mg; *b*, 2.2 mg; *c*, 1.8 mg. For Figs 1–3, the rate of heating in an oxygen-free nitrogen atmosphere is 3.6°C s^{-1} ; sample grain-size *d* is $45 < d < 106\ \mu\text{m}$; the glow is seen by a quartz-window photomultiplier (EMI 6256 SQ) through an Ilford broad-band (375–530 nm) filter no. 622 and plotted on a chart recorder by a sensitive electrometer; black-body radiation has been subtracted from the glow curve.

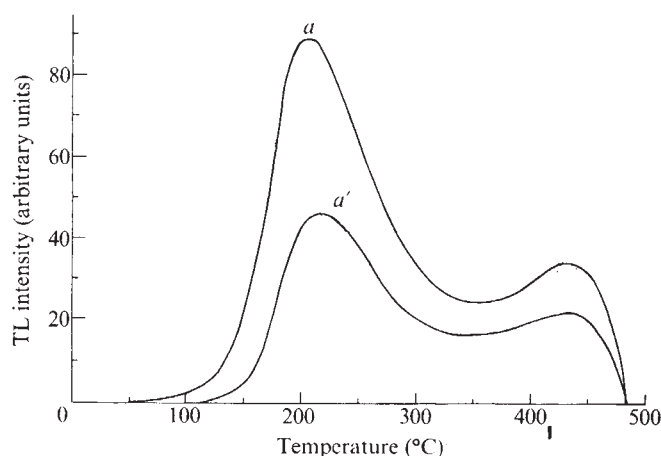


Fig. 2 Fading of the 'freezer counterpart' sample 76241,23 (curve *a'*), kept at room temperature for ~3 yr (date of readout, 26 January 1976), compared to the 'freezer sample' 76240,22 (curve *a*), kept at -20°C from 11 January 1973–22 March 1974, and at CO_2 -snow (-78.5°C) or liquid-nitrogen (-196°C) temperatures ever since. Most of the fading appears to be 'anomalous' (that is, not obeying the temperature-dependent equations). Sample weights: *a*, 1.6 mg; *a'*, 1.8 mg.

freezer counterpart samples over ~3 yr between sample retrieval and our experiments, natural-fading and isothermal-annealing studies have been carried out on the samples. Figure 2 shows the natural TL outputs at the beginning of 1976 from the freezer sample (76240) and its room-temperature counterpart (76241). The degree of fading, especially in the high-temperature region, is surprisingly high, suggesting anomalous fading³. In Table 1 we record the degree of fading observed in the two counterpart samples 76241 and 72321, after 3 yr of storage at room temperature, by comparing the natural TL surviving in three consecutive readout-temperature intervals of interest to us with the corresponding amounts in the freezer samples 76240 and 72320.

Dose response and inferred natural dose

For various calculations it is necessary to know the natural dose retained by each sample and the characteristics of the growth-to-saturation curves for them. In Fig. 3 we show a typical set of dose-response curves resulting from the artificial irradiation of a sample with ^{60}Co γ rays. The TL intensity from the sample (the shaded sample counterpart 76241, 23, 'sample *a'*') was integrated over three consecutive readout-temperature intervals, 216–306 $^{\circ}\text{C}$ (area I), 306–378 $^{\circ}\text{C}$ (area II), and 378–486 $^{\circ}\text{C}$ (area III), and the resulting 'TL glow areas' are separately plotted. By extrapolating such curves backward, we can infer the natural dose retained in each temperature interval by the sample. Similar curves have been obtained for the sunlit sample 76261, 25 ('sample *b'*').

From the growth curves we can also graphically determine the 'half dose' $R_{1/2}$, so termed² from analogy with half life in a growth-to-saturation curve in radioactivity. This is the dose required to fill half the remaining traps at any stage of irradiation, assuming the growth law

$$n = N(1 - \exp(-0.693 R/R_{1/2})), \quad (1)$$

where n is the number of filled traps for a total dose R (including the natural dose) and N is the total number of available traps of a given energy depth.

Theoretical framework

To determine the effective temperature and length of storage of the various samples in their lunar environment, we need to know the trapping parameters E ('trap depth', usually in eV) and s ('frequency factor', in s^{-1}) of the levels responsible for the observed TL. We have determined these parameters by using the 'initial rise method'⁴, and record them and other data needed in our calculations in Table 2.

In our calculations, we have assumed the trap-filling process to obey the 'first-order kinetics' of Randall and Wilkins⁵, and followed the general procedure of ref. 2. The following are the essential equations governing the radiation-filling and thermal-emptying of TL traps in generalised environmental conditions.

For a sample with a total of N traps, each of depth E and frequency factor s , irradiated with a dose rate r while being held at an absolute temperature T , the following differential equation holds

$$dn/dt = -\lambda n + (N-n)rp \quad (2)$$

where n is the number of filled traps at time t , λ is the decay constant at temperature T , and p is the probability per unit dose of filling one of the above traps. For parameters λ and p

$$\lambda = \tau^{-1} = 0.693/\tau_{1/2} = s \exp(-E/kT) \quad (3)$$

where τ and $\tau_{1/2}$ are the mean and the half lives, respectively, of the trapped electrons at temperature T , and

$$p = 0.693/R_{1/2} \quad (4)$$

where $R_{1/2}$ has been defined by equation (1).

The general solution of equation (2) is given by

$$n = \frac{Nrp}{\lambda + rp} \{1 - \exp(-(\lambda + rp)t)\} + n_0 \exp(-(\lambda + rp)t) \quad (5)$$

where n_0 is the number of filled traps at time $t = 0$.

There are two cases of special interest to us. First, if all traps are initially empty (for example, when trap-filling first starts in the sunlight), so that $n_0 = 0$, equation (5) is reduced to

$$n = \frac{Nrp}{\lambda + rp} \{1 - \exp(-(\lambda + rp)t)\} \quad (6)$$

Table 1 Fading of TL in the room-temperature 'counterparts' over a period of 3 yr by comparison with the 'freezer samples'

Sample	Area I (216–306 $^{\circ}\text{C}$)		Area II (306–378 $^{\circ}\text{C}$)		Area III (378–486 $^{\circ}\text{C}$)	
	TL mg^{-1} *	Fading	TL mg^{-1}	Fading	TL mg^{-1}	Fading
76240,22 ('freezer sample')	37.5		15.0		22.5	
76241,23 ('counterpart')†	20.0	47%	7.8	48%	12.8	43%
72320,4 ('freezer sample')	7.2		11.7		18.9	
72321,3 ('counterpart')	4.45	38%	8.3	29%	14.5	24%
76261,25 (sunlit sample)‡	0.68		1.82		7.27	

* TL (integrated over the temperature interval shown in each case) is expressed in arbitrary units, but has been normalised for sample weight.

† Separate studies on the fading of freshly produced TL in sample 76241, kept at room temperature, were carried out to estimate the fading of TL in the 'freezer samples' themselves as a result of the first ~3 weeks of 20°C storage of all samples at LRL in December 1972. This effect has been ignored in the above table (see ref. 6 for details).

‡ The values for the sunlit sample are recorded for completeness.

Table 2 Values of useful parameters for the shaded sample (*a*) and the sunlit sample (*b*) related to calculations for the temperature and duration of the boulder shadow

Parameter	Sample <i>a</i> (76241,23; 'freezer counterpart')			Sample <i>b</i> (76261,25)
	TL area I (216–306 °C)	TL area II (306–378 °C)	TL area III (378–486 °C)	TL area III (378–486 °C)
Trap depth E (eV)	1.046 ± 0.05	1.134 ± 0.07	1.439 ± 0.06	1.72 ± 0.06
Frequency factor s (s^{-1})	$\sim 5 \times 10^8$	$\sim 2 \times 10^8$	$\sim 1 \times 10^9$	$\sim 2 \times 10^{11}$
Peak temp. T_p (°C)	279	351	450	441
N/n_{eq} (or N/n)*	7.41	4.58	3.31	3.85
Half-dose R_i (krad)	500	520	520	340
Storage temp. T_1 (K)	256	256	256	371
Length of storage t_1 (yr)†	(calculated)	(from area I)	(from area I)	(calculated)
	—	3.98×10^4	5.65×10^4	∞
		(assuming 48% fading)	(assuming 43% fading)	
Mean life τ (T_1) (yr)	2.44×10^4	3.50×10^6	7.16×10^{11}	3.85×10^4 (at $T_1 = 371$ K)
Mean life τ (20 °C) (yr)	62	5.90×10^9	1.49×10^8	6.48×10^{10}

* These are the values of N/n (uncorrected for fading) as calculated from the heights of the TL dose-response curves (compare Fig. 3) at saturation, and at 0 artificial dose, respectively. In the case of area I (sample *a*) and area III (sample *b*), $n = n_{eq}$ (the corresponding traps having reached dynamic equilibrium in nature).

† These values of t_1 result from equation (11), with $r = 10$ rad yr $^{-1}$, after a fading correction for the relevant TL area for sample *a* (Table 1) has been applied to the measured N/n value shown in Table 2.

$$= \frac{N}{(1 + R_i/r\tau_i)} \{1 - \exp(-0.693rt/R_i)\exp(-0.693t/\tau_i)\} \quad (6')$$

Second, when the radio-thermal environment changes at time t_0 , say, (for example, the boulder arrives), such that the number of filled traps at time $t = t_0$ is n_0 , equation (5) holds, except that the values of the relevant parameters are changed to r' , p' and λ' , and t may be replaced by t_1 such that $t' = t - t_0$. Our chief interest, however, is in the case where the time before the arrival of the boulder is very large, so that $t_0 \rightarrow \infty$. The value of n_0 to be used in the modified form of equation (5) is then reduced, by substituting t_0 for t in equation (6) and then setting $t_0 \rightarrow \infty$, to

$$n_0 = Nrp/(\lambda + rp) \quad (7)$$

The modified form of equation (5), after the arrival of the boulder, thus becomes

$$n = \frac{N r' p'}{\lambda' + r' p'} \{1 - \exp(-(\lambda' + r' p')t_1)\} + \frac{N r p}{\lambda + r p} \exp(-(\lambda + r p)t_1) \quad (8)$$

A number of interesting limiting cases of equations (6, 6') exist, details of which is given elsewhere⁶. Here we confine ourselves to two cases of immediate relevance. Thus

(i) for either $rt \gg R_i$, or $t - \tau_i$

$$n_{eq} \rightarrow \frac{N}{1 + (R_i/r\tau_i)} \equiv \frac{N}{1 + (\lambda/rp)} \quad (9)$$

and if, in addition $r\tau_i \gg R_i$
 $n_{eq} \rightarrow N$ (10)

where, in equations (9) and (10), n_{eq} represents the dynamic-equilibrium, or steady-state, value of n reached either as a result of giving a massive dose ($rt = R \gg R_i$) to a sample, or by filling the traps for a very long time t compared to τ_i at the temperature concerned; and

(ii) for $\lambda \ll rp$, that is, $r\tau_i \gg R_i$

$$n \rightarrow N(1 - \exp(-rt p)) - N(1 - \exp(-0.693rt/R_i)) \quad (11)$$

The values of N and R_i in equation (11) are usually obtained by employing such a massive dose rate r in the laboratory that there is virtually no thermal drainage during the irradiation.

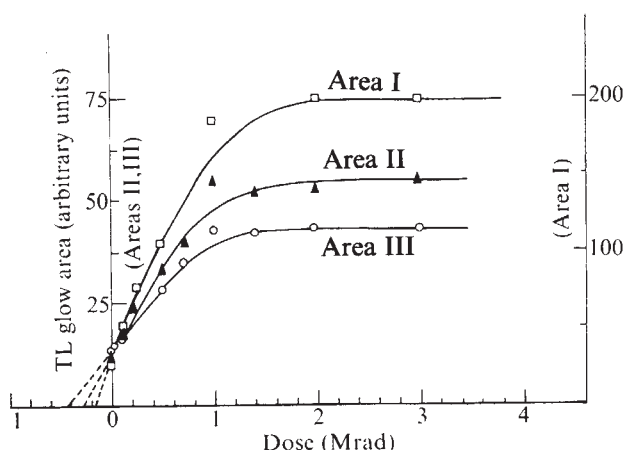
Determination of temperature and duration of the boulder shadow

We now determine the effective temperature of storage of the 'permanently shadowed' sample (76240 or 76241, 'sample *a*') and the length of its storage in the boulder shadow, by comparing the TL characteristics of that sample with those of the 'sunlit sample' (76261, 'sample *b*') and making use of the foregoing theoretical framework.

From equation (9), which applies to the case when dynamic equilibrium has been reached in nature (so that $rt \gg R_i$), we obtain, using equation (3), the value of the prevailing (or 'storage') temperature as

$$T = \frac{E/k}{\ln \left[\frac{sR_i}{0.693r(N/n_{eq} - 1)} \right]} \quad (12)$$

Fig. 3 TL response of sample *a* ('freezer counterpart' 76241,23) to γ -ray dose from a $\sim 3,500$ Ci ^{60}Co source (dose uncertainty, $\pm 3\%$). The TL has been integrated in each case over the three consecutive readout intervals termed area I (216–306 °C; $\square$), area II (306–378 °C; $\blacktriangle$) and area III (378–486 °C; $\circ$). Notice the higher scale (at right) for area I. From each of the curves the corresponding values of n/N (ratio of the ordinates at artificial dose = 0, and at saturation), and half-dose R_i (required to fill half the remaining traps at any stage) can be determined. Similar curves were obtained for sample *b* (the sunlit sample 76261,25); figure not shown.



where N/n_{eq} is the ratio of the saturation TL (obtained from a separate experiment) to that found in the natural sample. If the natural dose-rate r is known, T can be calculated by determining the values of E , s and R_i for the relevant trapping level. We assume⁷ a value of $r = 10 \text{ rad yr}^{-1}$ for unshaded lunar samples. As r occurs in the logarithmic term, even a substantial change (say reduction by a factor of 2) in its value after the arrival of the boulder has little effect on the calculated value of T . This also applies to the effects of fading corrections (discussed below) on N/n_{eq} .

In Table 2 we record the experimental values of all the parameters that are needed in our calculations. Our calculations involve the following steps:

(1) We assume in the case of the sunlit sample (*b*) that dynamic equilibrium has been reached in filling the traps corresponding to the TL readout area III (378–486 °C, with $E = 1.72 \text{ eV}$), that is, $t_i \gg \tau_i$ in sunlight. By using the values of the various parameters given in Table 2 (and after applying a 43% fading correction to N/n_{eq} by inference from the corresponding value for sample *a* in Table 1), we obtain from equation (12) the lunar storage temperature $T = 371 \text{ K}$ for the sunlit sample.

(2) We then calculate from equation (12) the storage temperature T_1 in the shade by assuming that equilibrium has been reached in TL area I of the shaded sample (216–306 °C; $E = 1.046 \text{ eV}$), that is, $t_i \gg \tau_i$ of the trap at temperature T_1 (compare Table 2). After applying a 47% fading correction to n_{eq} in the 'counterpart' sample, (compare Table 1), we find that the temperature in the boulder shadow is $T_1 = 256 \text{ K}$.

(3) At $T_1 = 256 \text{ K}$, the values of τ_i for the traps corresponding to TL area II (306–378 °C; $E = 1.134 \text{ eV}$) and area III (378–486 °C; $E = 1.439 \text{ eV}$) of the shaded sample are found to be such that the condition governing equation (11) is fulfilled ($\tau_i \gg R_i$; compare Table 2). We can therefore solve that equation to obtain the length of storage t_1 in shade from either of these TL areas, using the known values of n/N . This implies that the natural TL in areas II and III of sample *a* is well below the corresponding saturation values.

Assuming the values for the amount of fading observed for TL areas II and III in the 'counterpart' sample *a* (namely 48 and 43%, respectively, compare Table 1), we obtain from equation (11) the following values for the length of storage, t_1 , in the boulder shadow:

from area II (sample *a*): $3.98 \times 10^4 \text{ yr}$; from area III: $5.65 \times 10^4 \text{ yr}$.

These values are based on a dose-rate $r = 10 \text{ rad yr}^{-1}$; if that rate is halved in the shadow of the boulder, the length of storage would be doubled, and so on.

Correction factor for 'anomalous' fading

The calculations based on the foregoing framework are found to be very sensitive to the values of the parameters for the various samples and TL areas that are fed into the equations. We mention in passing that the lengths of storage in the shade, as calculated from TL glow areas II and III of sample *a*, can be made to agree if one assumes an empirical relation for 'anomalous fading'³, namely: fading $\propto 1/E$, where E is the trap depth. This leads to the following values of anomalous fading in areas I, II and III of sample *a*: 68, 62 and 49%, respectively (and for area III in sample *b*: 41%). If one assumes such fading to have taken place in the shadowed sample since retrieval, the duration of the boulder shadow is obtained (from both areas II and III of sample *a*) as $t_1 = 6.50 \times 10^4 \text{ yr}$ (for $r = 10 \text{ rad yr}^{-1}$).

Conclusion

That we have been able to calculate the value of the duration of the shadow cast over our sample *a* (namely $\sim 4.0\text{--}6.5 \times 10^4 \text{ yr}$), and hence the time of the arrival of the boulder at its pre-

sent location, has important implications for soil mechanics on the surface of the moon. Mitchell *et al.*⁸ refer to over 300 tracks "made by boulders rolling, bouncing and skidding down lunar slopes". The Apollo 17 mission provided the first opportunity to study closely these interesting features which were very evident on the Taurus-Littrow hills. The mechanisms for setting the boulders in motion are not well understood, but could include cyclical thermal expansion and contraction, impact cratering and seismic events. Our estimation of the time-scale involved in at least one such event could be helpful in clarifying the picture.

In considering the duration of boulder shadow as calculated from the preserved natural TL in the shaded soil, it could be instructive to consider the role of dust movement on the lunar surface. Criswell⁹ postulates the migration of electrically charged micrometre-sized grains from the sunlit areas towards dark or partially illuminated areas. From 'horizon glow' observations made at the time of lunar sunset, Criswell calculates a value of mass-flow rate of $\sim 10^{-3} \text{ g cm}^{-2} \text{ yr}^{-1}$ for the surface dust, corresponding to a layer turnover rate of $\sim 6 \mu\text{m yr}^{-1}$. Such a rate of inflow would deposit a layer $\sim 30 \text{ cm}$ thick in 50,000 yr under the boulder. That this is not the case is clear when one considers the top $\sim 5 \text{ cm}$ of the shadowed soil (the maximum depth of our scooped sample *a*). A thickness of 5 cm would, at the above rate, be deposited in $\sim 8,000 \text{ yr}$, yielding an average age of only $\sim 4,000 \text{ yr}$ inside the shadow, whereas our calculated length of storage in the shade for sample *a* is 40,000–65,000 yr. Assuming the top 1 cm in the shade to be of external origin, the Criswell rate of inflow must be reduced by a factor of ~ 40 . (This results in the duration of the boulder shadow being revised upwards from $6.0 \times 10^4 \text{ yr}$ to $6.67 \times 10^4 \text{ yr}$ to yield the observed amount of glow, assuming a homogeneous mixing of the top 5 cm of soil during and since its retrieval.)

The value of the storage temperature in the boulder shadow obtained by us (256 K) seems to be high when one considers the conductive processes on the Moon. Values as low as $\sim 25\text{--}100 \text{ K}$ can be obtained by using the lunar heat-flow data of Langseth *et al.*¹⁰ or our own conductivity data¹¹, but such calculations are strongly model dependent. Our value in the shade is quite close to the mean equilibrium temperature on the lunar surface ($\sim 240 \text{ K}$). Imperfect shadowing of the sample (which came from only $\sim 0.5\text{--}1 \text{ m}$ inside the boundary of the shadow) and soil mixing could explain the rather high value of the effective storage temperature in the shadow of the boulder estimated by us. Our calculation depends critically on the value n_{eq}/N (the fraction of total traps filled at equilibrium) in the TL glow area I of sample *a*. If, as a result of possible exposure to sunlight during the retrieval procedure and/or the high-temperature ($\geq 100^\circ\text{C}$) handling of the sample during EVA on the lunar surface, or because of a greater degree of anomalous fading than we have allowed for, the initial value n_{eq}/N was higher than that used in our calculations, the temperature in the shade would have to be revised downward. Similarly, if dynamic equilibrium had not as yet been reached in trap-filling for area I of sample *a* (owing, say, to a relatively short storage time in the shade), then equation (12) would not hold, and the value of storage temperature obtained by us would be an overestimate.

Our investigations have, however, been limited to the measurable effects in the context of information currently available, and the values obtained by us are the best estimates that can be made at present for the temperature and duration of the shadow of the lunar boulder concerned. Further refinement of the data may be possible in our future work which involves the simulation of the lunar irradiation conditions.

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Climate periods in tree, ice and tides

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Ten climate periods found in stable isotope ratios of oxygen and hydrogen, measured in 1,800 yr of Japanese cedar rings, agree with climate periods found in 800 yr of Greenland ice and with periods computed from the tidal stresses of the Sun–Moon–Earth system, and with periods found in the ^{14}C record of the bristlecone pine sequence of southern California. The Greenland oxygen ratios have previously been found to have opposite phase to the ^{14}C ratios of the bristlecones, and we have found also an opposite phase between oxygen and hydrogen ratios in the Japanese cedar on the one hand and ^{14}C in bristlecones on the other hand.

VARIATIONS of the oxygen and hydrogen isotope ratios have been measured in a Japanese cedar ring sequence of about 1,800 yr (see Fig. 1 and ref. 1). The Fourier transforms of these isotope ratios against age, into power against reciprocal periodicity, show the 10 periods enumerated in Table 1. Eight

of these periods are evidenced in variations of the oxygen isotope ratio in Greenland ice². Three of these 10 periods are evidenced in tidal stresses calculated for the Sun–Moon–Earth system³. The periodicities evaluated from these four sets of data agree with each other within statistical accuracy.

Why do the periods lie between 50 and 300 yr? The answer is to be found in the nature of sampling. Our cedar sequence was sampled integrating over 5 yr at a time and therefore cannot reliably show evidence for periods of less than about 50 yr. To look for periods of < 50 yr in a sequence of 5-yr samples or to look for periods of > 300 yr in a sequence of 1,800 yr is probably meaningless. Such periods may appear in Fourier transforms but they do not necessarily represent reality.

To understand this we generated random signals of values 0 or 1, and assigned them to 10-yr intervals in 1,000-yr hypothetical sequences, and made their Fourier transforms. In three such computer experiments we always obtained a period of about 20 yr as an artefact of the sampling interval. In part it occurs because short sequences of random numbers (say 100 numbers) are not really random, and in part because it

Fig. 1 Stable isotope ratios of oxygen and hydrogen measured in Japanese cedar, AD 140–1950, in whole wood samples, integrating over 5 yr each. PDB, Pee Dee Belemnite, a fossil shell used as standard; SMOW, standard mean ocean water.

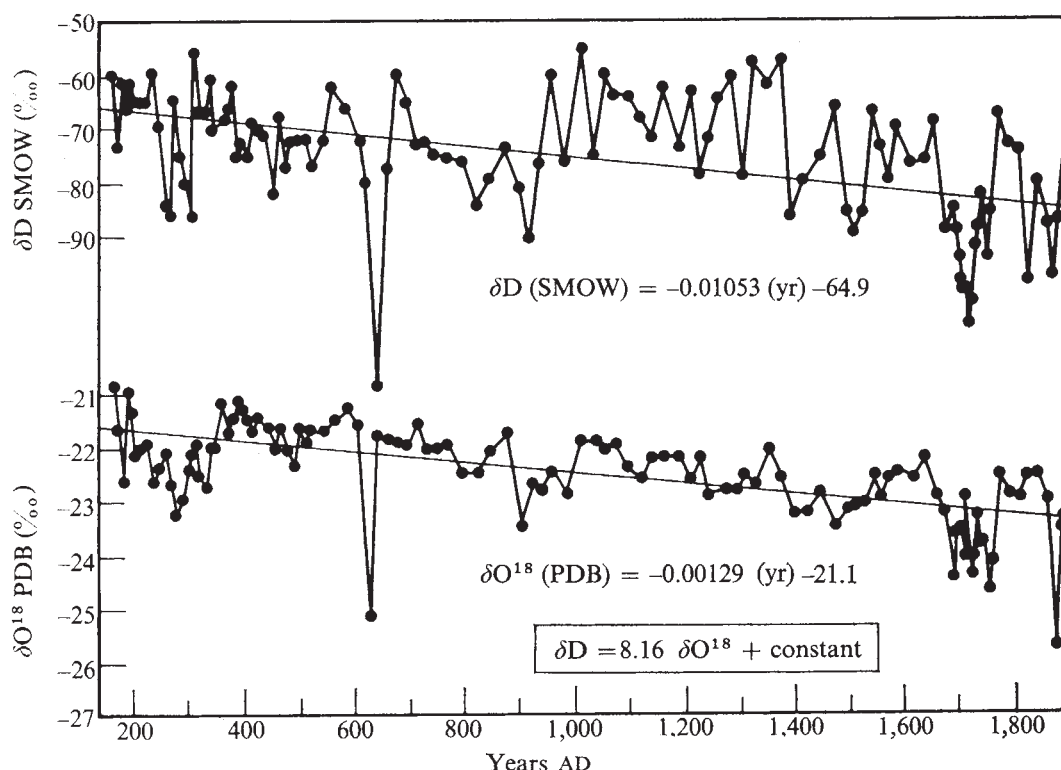


Table 1 Periods and respective powers evaluated in Fourier transforms of oxygen and hydrogen isotope ratios in a Japanese cedar AD 140–1950 (ref. 2) and in oxygen isotope ratios in the Greenland ice cap, AD 1200–1950

$\delta (^{18}\text{O}/^{16}\text{O})^*$		$\delta (\text{D}/\text{H})^*$		$\delta (^{18}\text{O}/^{16}\text{O})$ (ice and tides) [†]	
Period (yr)	Relative power	Period (yr)	Relative power	Period (yr)	Relative power
54 $\pm$ 3	0.58	52 $\pm$ 5	1.00	55 (b)	—
57 $\pm$ 3	0.65	57 $\pm$ 3	0.60	62 (b)	—
59 $\pm$ 3	0.70	61 $\pm$ 2	0.40	68 (b)	—
69 $\pm$ 3	1.50	66 $\pm$ 4	1.64	78 (b)(a)	strong
92 $\pm$ 2	0.52	87 $\pm$ 3	1.00	86 (a)	—
96 $\pm$ 2	1.29	95 $\pm$ 2	2.18	100 (b)	—
154 $\pm$ 6	2.58	153 $\pm$ 7	2.18	142 (a)	—
174 $\pm$ 7	1.29	176 $\pm$ 7	2.00	179 (a) 181(b)	strong
202 $\pm$ 7	1.45	205 $\pm$ 9	1.70	—	—
271 $\pm$ 11	1.80	273 $\pm$ 20	1.64	285 (a),(b)	—

* Data from ref. 1.

[†] Data from ref. 2.(a) Denotes tidal³ and (b) denotes ice core². At about the 86-yr period the tidal data and ice core data are almost exactly 180° out of phase.

provides an alias of the sampling interval (see ref. 4 and consider the following example):

Let each sample integrate over $\Delta t = 10$ yr in a sequence of 100 samples corresponding to 1,000 yr. Such a short sequence, even if chosen randomly, produces artefactual signals at periods of 10, 20, 30, ... yr in the power spectrum, as we have found in computer experiments with random numbers. Then the sampling frequency, $f_N = (1/2 \Delta t) = 1/20 \text{ yr} = 0.05$ cycles per yr

combines with the artefactual signal frequency, $f = 1/20 \text{ yr} = 0.05$ cycles per yr

to produce the following alias signals, $f, 2f_N \pm f, 4f_N \pm f, \dots$

equivalent to 0.05 cycles per s, (0.1 $\pm$ 0.05) cycles per s, (0.2 $\pm$ 0.05) cycles per s, ... so that the original signal at 20 yr is reinforced by the alias signal at $1/(0.1 - 0.05) = 20 \text{ yr}$.

If instead, the sampling period is 5 yr, corresponding to $f_N = 1/5 = 0.2$ cycles per yr the artefactual signals lie at $f^{-1} = 5, 10, 15, \dots \text{ yr}$ and the aliased signals are all at periods of $\leq 10 \text{ yr}$.

Blackman and Tukey (ref. 4, pp. 31–32) warn us "... frequencies above f_N usually contribute some power. Each frequency, no matter how high, is indistinguishable from one in the band from 0 to f_N ." Fortunately, there is little power in our D/H and $^{18}\text{O}/^{16}\text{O}$ spectra at high frequencies so we feel safe from aliasing at periods greater than about 20–30 yr. Thus we are in conformity with the remark (ref. 4, p. 52) that "It is likely that (power) decreases substantially as f goes from 0 to (the sampling frequency). If it does not, then aliasing is likely to have been serious, and satisfactory analysis at this spacing may be impossible." So we seem to be safe from aliasing for periods greater than 50 yr.

If we are guided by ref. 4, p. 11, the maximum oscillation which we should search for in a sequence of 1,800 yr is "a moderate fraction (perhaps 5–10%) of the length of the record" or 90–180 yr, but we shall stretch this to 300 yr because of the agreements among the four sets of periods shown in Table 1. Thus where we are cautioned⁴ to study only periods which can oscillate more than 10–20 times in our data sequence, we have dared to take seriously periods which can oscillate 6 times or more in 1,800 yr.

With respect to a DC component, ref. 4, pp. 48–49 states, "All the DC power belongs in the very lowest frequency band ...". We took the precaution, however, to subtract the DC component from both the D/H and $^{18}\text{O}/^{16}\text{O}$ data (as in ref. 4, p. 48). Thus as shown in our Fourier transforms¹ there is very little power in periods of more than 400 yr. Furthermore, we have excluded periods of $> 300 \text{ yr}$ from our present considerations because they can oscillate less than 6 times in 1,800 yr.

The ratio of peak height to background in computer experiments on relatively short sequences of random numbers gives a way to assess what is the background caused by 'noisiness' of the isotope data. The peaks listed in Table 1 are significantly above background as shown in ref. 1, Fig. 7.

The validity of a 300-yr period in 1,800 yr of data can be tested by another computer experiment. Specifically one may

generate a 300-yr periodic sine function for 1,800 yr, mix it with sine functions of shorter periodicities, and transform the mixture to show that the 300-yr signal is evidenced. Similar computer experiments were performed to see if short periods of 54, 57 and 59 yr are meaningfully separable. Our results indicate that they are. The measurements of Dansgaard *et al.*² were made on samples integrating over about 14 yr each, in an ice sequence of about 800 yr.

Thus, are periods between 50 and 300 yr in the ice cap, and even in the tree, representative of real climate variations? The remarkable agreement between the three sets of data suggests that they are. The agreement is expected, and explained, of course, because the ice cap is storing rain water and the tree is likewise storing rain water by changing it chemically to wood.

The evidence that the tree is storing a record of the climatically induced changes in rain water is that the least squares correlation of D/H against $^{18}\text{O}/^{16}\text{O}$ in the 1,800-yr sequence yields the relation¹

$$\delta \text{D}/\text{H} = 8 \delta ^{18}\text{O}/^{16}\text{O} + \text{constant}.$$

The empirical slope of 8 is the same as in world-wide rain water within experimental error⁵.

Thus the question of whether hydrogen and oxygen isotopes, bound in wood made in previous years, can be exchanging with sap of the current year, seems to be answered conclusively in the negative¹; specifically, rings of previous years are sealed against exchange. If both O and H in OH groups of wood exchange with sap water, the slope cannot be 8, because there are twice as many H atoms bound to carbon, and these do not exchange.⁶

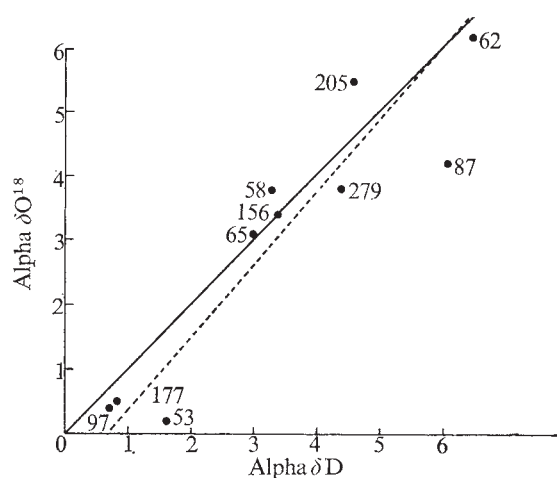
Fig. 2 Relative phases of ten climate periods evaluated for D/H, plotted against relative phases evaluated for $^{18}\text{O}/^{16}\text{O}$. Each point is marked by its respective period in yr. Phases are in radians. The dotted line is the best fit straight line, for which $\alpha \delta ^{18}\text{O} = 1.13 (\alpha \delta \text{D}) - 0.771$, and $r = 0.922$.

Table 2 Periods and their relative amplitudes and phases

T (period)	A (amplitude)	Alpha (α)	T (period)	A (amplitude)	Alpha (α)
279	3.51	4.40	287	3.29	3.81
205	3.21	4.57	205	3.09	5.46
177	3.71	0.77	179	2.70	0.53
155	3.96	3.37	157	3.96	3.37
97	3.91	0.73	96	2.73	0.39
86	2.42	6.09	88	1.75	4.19
65	3.26	3.02	70	2.90	3.06
62	1.64	6.47	66	1.49	6.21
58	2.06	3.27	57	2.04	3.78
53	2.63	1.56	54	1.84	0.21

Data based on Fourier transforms of our oxygen and hydrogen isotope ratios in a Japanese cedar AD 140–1950.

The remarkable agreement between our tree records of D/H and $^{18}\text{O}/^{16}\text{O}$, and the ice record of $^{18}\text{O}/^{16}\text{O}$, shows itself in yet another way. We have found the D and ^{18}O ratios for the Japanese cedar to be significantly correlated in opposite phase to the ^{14}C variations in the bristlecone pines of Southern California.⁷ Similarly Dansgaard *et al.*² found the ^{18}O record in Greenland ice to be significantly correlated in opposite phase with bristlecone ^{14}C .

We have also made a Fourier transform of the bristlecone pine ^{14}C record and find the following periods: 96, 183, 202 and 272 yr, in agreement with the longer periods in Table 1. Periods of <100 yr are probably not meaningful in Suess' measurements⁸ because of his 30-yr sampling period.

The Fourier transform also evaluates amplitudes and phases of the periods evidenced in the measurements. For the isotope ratios measured in the Japanese cedar, these are listed in Table 2 for oxygen and hydrogen, and are in agreement with each other. A graph of the hydrogen phases versus the oxygen phases shows this agreement (Fig. 2).

We conclude from these observations—(1) The climate variations in Greenland, southern Japan, and southern California have had the same periodicities for the last 800 yr or more. Climate variability therefore seems to be a global phenomenon.

(2) The calculation of Dansgaard *et al.*² of the age of ice against depth in the Greenland ice cap seems to be correct, with an error of not more than a couple of years, at least over the last 800 yr. The Japanese cedar rings have been dated by ring counting and by about 50 radiocarbon dates in the 1,800-yr sequence⁹; this serves to calibrate the calculated age of the ice by means of the present comparison of climate periodicities in the tree and in the ice¹⁰.

Our conclusions depend on measurement of hydrogen and oxygen isotope ratios in a single tree: substantiation requires measurement of other tree sequences.

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Comparative neutron small-angle scattering study of small spherical RNA viruses

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Small-angle neutron scattering from solutions of small RNA viruses has been used to study protein–nucleic acid organisation. In the five viruses investigated the RNA is confined to a sphere of about 100 Å radius, with a central hole (with one possible exception). The interpenetration of RNA and protein varies with viruses and seems to be related to the nature of the forces stabilising the virus.

THE basic principles of the architecture of viral protein shells are now well established¹, and the detailed assembly of protein subunits in the capsids of several small RNA viruses has been determined by electron microscopy and X-ray diffraction^{2,3}. Very little is known, however, about the nucleic acid. Indeed, electron microscopy provides no information on the internal structure of small viruses, and low-resolution X-ray diffraction does not allow unambiguous location of the RNA, which

represents, at most, 35% of the total mass. Klug *et al.*⁴ compared low-resolution X-ray diffraction patterns of Turnip Yellow Mosaic Virus (TYMV) virions and the associated empty capsids, and attempted to modify the relative contributions of protein and RNA by increasing the solvent electron density by addition of salt. The contrast variation method has also been used by Harrison⁵ and Zipper *et al.*⁶. It is limited, however, because high ionic strengths may dissociate virions or modify their structure, and it is practically impossible to raise the density of the solvent to the level at which it matches that of compact RNA.

Neutron scattering by virus solutions provides a straightforward alternative for obtaining low-resolution information about the viral nucleic acid, and utilises the fact that H_2O – D_2O mixtures can be made with scattering powers that match either RNA or protein^{7,8}, making it much easier to distinguish between protein and hydrated RNA at low resolution. The possibility that D_2O may affect the stability or the structure of the virus

Table 1

Name	Protein subunit MW	RNA content (MW) of the virion	Total MW
Turnip Yellow Mosaic Virus (TYMV)	20,130	1.9×10^6	5.53×10^6
Southern bean mottle virus (SBMV)	29,390	1.4×10^6	6.69×10^6
Cucumber mosaic virus (CMV)	26,000	$\sim 1.10 \times 10^6$ (multigenome)	5.78×10^6
Brome grass mosaic virus (BMV)	20,300	$\sim 1.05 \times 10^6$ (multigenome)	4.70×10^6
MS2	13,750	1.142×10^6	3.66×10^6

must be checked in each case, but D_2O is less likely to have an effect than high salt. The method is of general application and allows a simple systematic comparison between viruses. We report here the results of such a survey made on the five viruses,

all with triangulation number¹ $T = 3$ (180 subunits), listed in Table 1.

The viruses were selected to look for a possible correlation between stabilising forces⁹ and morphology. TYMV is characterised by very strong protein-protein interactions¹⁰, as shown by the existence *in vivo* of stable empty capsids. CMV, by contrast is stabilised mainly by RNA-protein interactions¹¹, being sensitive to ribonuclease and to high ionic strength. In bacteriophage MS2 both types of interactions are important¹². BMV belongs to a special category: at acid pH it is insensitive to ribonuclease and high salt and empty shells can be formed *in vitro*; at pH 6.5 it swells, becomes ribonuclease sensitive and dissociates in high salt¹³. The physical chemistry of SBMV is not yet well documented, but this virus is under study by X-ray crystallography^{14,15}. All viruses were dissolved in low molarity buffers (≤ 0.02 M).

Data collection and analysis

The details of the method will be described elsewhere¹⁶. It consists in collecting the small-angle diffraction pattern of the virus solution at various levels of contrast between virus and solvent, that is, at various solvent D_2O contents. The contrast, $\bar{\rho}$, is defined as the difference between the average scattering density of the virus and the scattering density of the solvent. A typical diffraction curve is shown in Fig. 1. This intensity curve can be put on an absolute scale by comparison with scattering from water and from the intensity scattered at zero angle by a dilute solution of a well-known virus (here TYMV).

A complete analysis of this set of curves gives the following information.

(1) Radius of gyration

For small values of the scattering vector s ($s = \sin \theta / \lambda$ where θ is the scattering angle and λ the neutron wavelength), the scattered intensity varies as

$$I = I(0) \exp\left(-\frac{4}{3}\pi^2 s^2 R_G^2\right)$$

The radius of gyration R_G varies with the mean contrast, $\bar{\rho}$. The relation between R_G and $\bar{\rho}$ is¹⁷

$$R_G^2 = R_{GV}^2 + \frac{\alpha}{\bar{\rho}} - \frac{\beta}{\bar{\rho}^2}$$

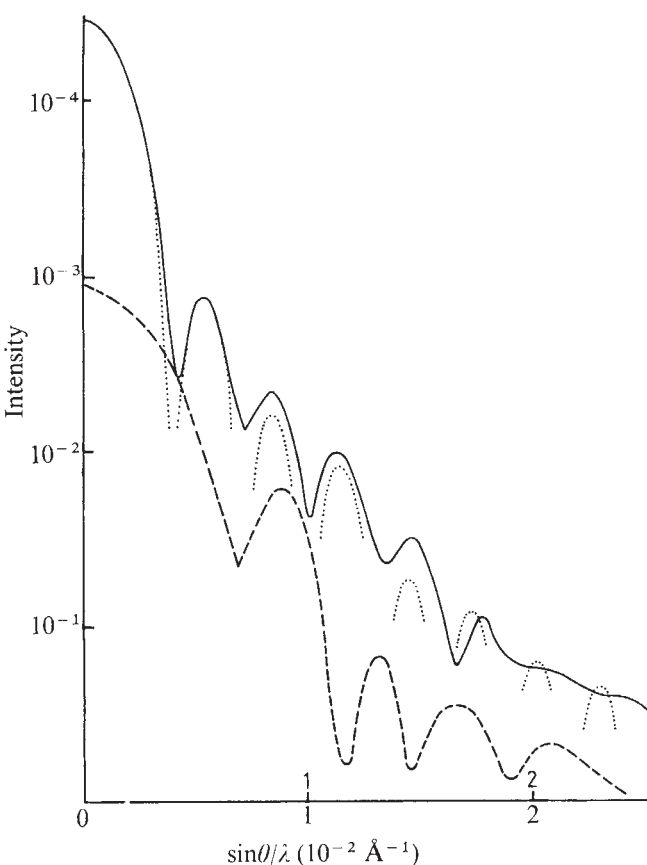


Fig. 1 Typical diffraction curves obtained for SBMV solutions, corrected for background (which is flat). —, Buffer solution (20 mM phosphate, pH 7) in H_2O . ----, Buffer solution with 43% D_2O . The large shift of the first maximum in 43% D_2O shows that the RNA is spread over a much smaller volume than the virus., Curve calculated for the uniform spheres model (Fig. 2).

Table 2

Virus	α_{exp}	R_{GV}	α_{cal}	Position of first subsidiary maximum			Relative intensity of the first maximum $I(0)/I_1$		
				in H ₂ O	in 43 % D ₂ O	in 68 % D ₂ O	in H ₂ O	in 43 % D ₂ O	in 68 % D ₂ O
TYMV	3,450	111	3068	6.43	8.91	5.79	107	60	28
SBMV	2,840	123	2530	6.27	8.91	5.79	85	16	36
CMV	1,680	127	2160	6.30	8.75	5.79	60	20	34
BMV low pH	2,870	113	2455	6.92	9.55	6.16	65	not measured	30
BMV high pH	4,720	134	4400	6.06	7.16	5.44	40		26
MS2	4,710	117	5600	6.53	8.59	6.05	46		20

R_{GV} in Å; α is dimensionless, α_{cal} is calculated as explained in the text.

Positions of intensity maxima are given in $\sin \theta / \lambda$ and in units 10^{-3} Å^{-1} .

I_1 , the intensity of the first maximum, is expressed by the ratio $I(0)/I_1$, where $I(0)$ is that of the central peak. For a solid sphere the ratio $I(0)/I_1$ is 135; it decreases for a hollow sphere with the size of the central hole, reaching 20 for a thin spherical shell.

Table 3 Results of our survey on five viruses

	R_{ext} (Å)	Protein peak position (Å)	RNA radius (outside) (Å)	Central hole (Å)	Capsid inside radius (Å)	RNA peak position (Å)	RNA peak density (Å ⁻²)	Protein peak density (Å ⁻²)	ρ protein in H ₂ O (calculated; Å ⁻²)
TYMV	143	117	103	< 30	~95–100*	~45	2.1×10^{-6}	2.50×10^{-6}	2.47×10^{-6}
SBMV	145	125	100	~30	~80–85 ~98 ~100	~55	1.5×10^{-6}	2.01×10^{-6}	2.34
CMV-D	145	117	100	~60	~80–85 ~80 ~80	~70	1.6×10^{-6}	2.10×10^{-6}	2.38
BMV low pH	130	110	95	~50	~80 ~80				
BMV high pH	152		130	~75					
MS2	135	115	100	~60	~105 ~100	~65	1.55×10^{-6}	2.50×10^{-6}	2.40

Capsid inside radius (a) From $I(0)/I_1$. (b) From Fourier transform.

*Data have also been collected on TYMV empty capsids, giving the same results as for TYMV protein shell¹⁶.

A linear variation of R_G^2 with $1/\bar{\rho}$ has been obtained for all the viruses investigated. The values of R_{GV} and α are listed in Table 2; β is zero within experimental error in all cases. R_{GV} is in first approximation the radius of gyration of a homogeneous particle with the same morphology as the virus, but spatial inhomogeneities of H-D exchange also contribute to it^{8,17}. A value of $R_{GV}^2 < 3/5 R_{\text{ext}}^2$ (expected for a uniform sphere), where R_{ext} is the outside diameter of the virus (see below), suggests the presence of a central hole, because the radius of gyration of a hollow sphere is larger than that of a solid sphere of the same diameter. α is the second moment of the density fluctuations around their average, at zero contrast between solute and solvent. These fluctuations are determined by the internal structure of the virus. The values of α agree well with those calculated for very simple models consisting of a sphere of RNA surrounded by a protein shell, with dimensions taken from Table 3. β should be zero if the centre of gravity of the scattering mass does not vary with contrast, for example, if the protein shell and the RNA have the same centre of gravity. The experimental data show this to be the case. A separation of 10–20 Å between centres of mass of RNA and protein would not, however, be detectable in our experiments.

(2) The dimensions of the virion and its RNA

The simplest way to analyse small-angle scattering curves from 'spherical' viruses is to find the dimensions of the homogeneous sphere (or hollow sphere) which best fits the innermost part of the diffraction pattern, in particular the position and the intensity of the first subsidiary maximum (Table 2). The non-spherically-symmetric components of the structure do not contribute to that maximum³ and a first set of values can be obtained by considering the virus to be composed of two spherical shells, each with uniform density, one for protein and one for RNA. Moreover, data have been obtained in 43% D₂O, where the diffraction pattern is dominated by the contribution of RNA, and 68% D₂O, where it is essentially due to protein. The radii obtained are self consistent. For instance the outside diameters deduced from the scattering in H₂O and 68% D₂O are the same, and the sizes of the central hole deduced from the data in H₂O and 43% D₂O. This first set of values can then be used to build models, which are refined using several subsidiary maxima. The values finally obtained (Table 3), are close to the first set. We have already seen that those values account well for the variation of the radii of gyration with contrast. The outside diameter is also confirmed by the Fourier transform of $I(s)$, which equals zero at the maximum diameter of the diffracting mass¹⁸. The values obtained for the outside diameter agree well with previous results for TYMV (ref. 4), BMV (ref. 20) and MS2 (ref. 6). The large intensities of the first subsidiary maxima (Table 2) strongly suggest the existence of large central holes in all of these viruses, with the possible exception of TYMV, where the hole (if any) is much smaller.

Another feature emerges from Table 2: with the exception of swollen BMV, at high pH, the first subsidiary maximum of the

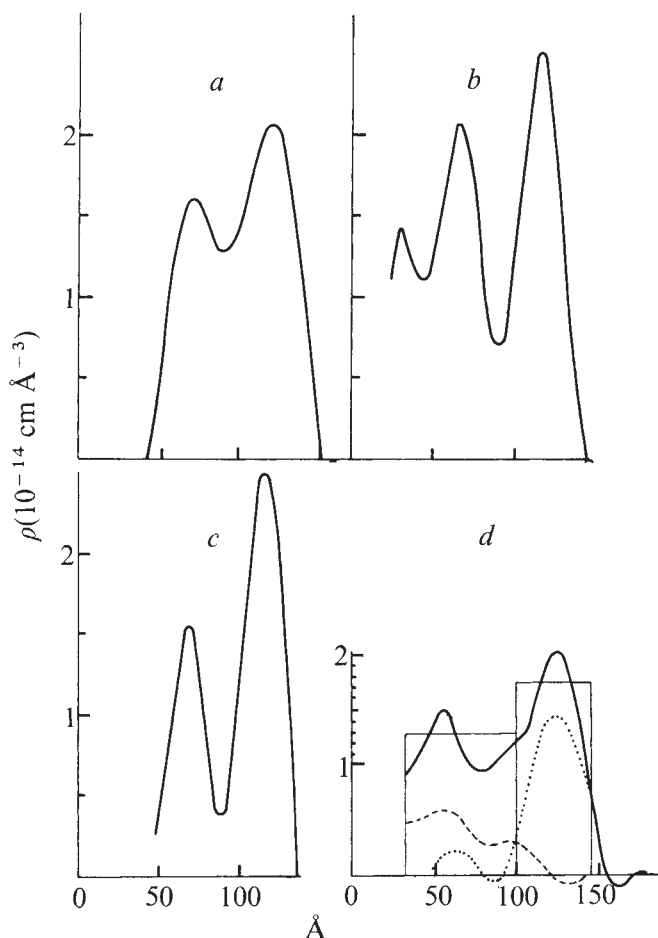
patterns dominated by the diffraction of RNA (in 43% D₂O) is at nearly the same position for all of these viruses and, corresponds to an overall diameter of the RNA region of about 100 Å.

(3) The radial distribution of protein and RNA

The radial scattering density $\rho(r)$ of the particles gives a more detailed picture. For an object with spherical symmetry,

$$\rho(r) - \rho_s = \frac{2}{r} \int_0^\infty s F(s) \sin 2\pi r s ds$$

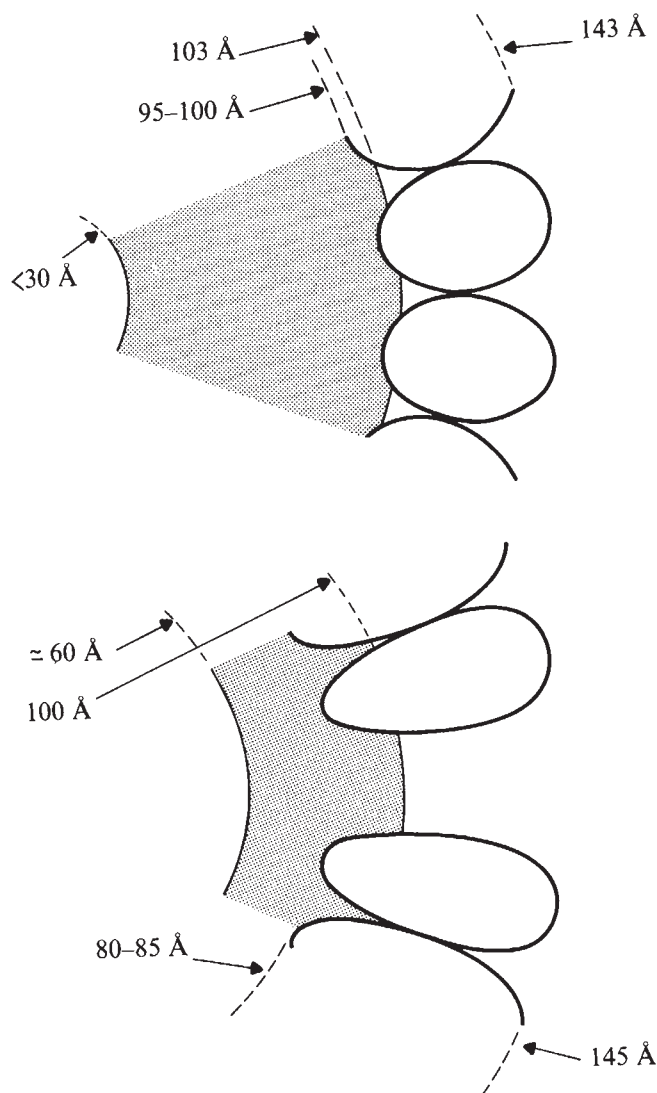
Fig. 2 Radial densities obtained by spherical Fourier transformation of structure factors deduced from scattered intensities: a, CMV in H₂O; b, TYMV in H₂O; c, MS₂ in H₂O; d, SBMV: —, in H₂O, ----, in 43% D₂O,, in 69% D₂O. The rectangles give the model used for the calculated curve (Fig. 1). All solvents were buffers of low molarity (0.02 M or below).



where ρ_s is the scattering density of the solvent. $F(s)$ is the square-root of $I(s)$, and its sign is assumed to change at each minimum of $I(s)$ as a consequence of the principle of minimum wavelength¹⁹. It is pertinent to compute $\rho(r)$ from data collected in H_2O and in buffers matching the contrasts of the protein and RNA moieties (that is, about 43 and 68% D_2O).

In Fig. 2 some of these radial densities are shown on an absolute scale. The variation with contrast of the protein part (the peaks at large radius), and of the RNA part (the more complex structure between the centre and the protein shell) confirms the attribution of the density to the corresponding moiety. These curves are deformed by cut-off effects in Fourier transforms and no reliable information can be obtained near the centre ($r < 50$ Å). The density at the maxima of the protein peaks, for instance in H_2O , is rather independent of the cut-off (Table 3). For TYMV and MS2 the experimental value of the density of this peak is that calculated for their coat protein, indicating a very tight packing of the subunits in the capsid. In

Fig. 3 Sketch of the organisation of protein and RNA in two viruses as deduced from neutron low-angle scattering data. The shaded area corresponds to RNA. *a*, TYMV. Protein molecules are represented in close contact. RNA does not penetrate deeply between subunits of the capsid. *b*, CMV. The shape of the protein shell has been chosen to fit the data of Table 3. The size of the holes in the capsid has been calculated assuming 32 large holes (in the centres of hexamers and pentamers of the capsid). In this model the total volume accessible to RNA is about 2.5×10^8 Å³; its average scattering density when this space is uniformly filled with CMV RNA is 1.60×10^{-14} cm Å³, in excellent agreement with the experimental value.



contrast, for CMV and SBMV the experimental values are somewhat lower, suggesting the presence of channels between subunits in the capsid. The position and width of the protein peak is also very well determined, both by the radial density in H_2O and in 68% D_2O (Table 3). Except for SBMV they agree with those obtained from the analysis of the position and intensity of the first subsidiary maximum.

For the RNA, which is located nearer the centre of the virus, the results are not so reliable. First, at contrast matching of the protein, the diffraction pattern has a fairly low intensity, and, except for SBMV, where a very concentrated sample was available, does not extend very far. Second, for a given resolution, Fourier transforms give less reliable results at smaller radii. The position of the RNA peak (Table 3) is correlated with the estimated size of a central hole as expected. The density of this RNA peak is also given in Table 3. Its accuracy is less than for the protein peak, but it is much lower than the density of pure RNA (4.04×10^{-6} Å⁻²), indicating that 50–60% of the volume is occupied by water, even in the region where the RNA is the most closely packed.

Discussion

The analysis of the neutron diffraction at small angle by spherical viruses in solutions of buffer containing different amounts of D_2O provides a consistent set of data, from which low-resolution models can be built.

The comparison between values obtained for the dimensions and the density of the two moieties contributing to those viruses show the following features:

- (1) All viruses studied in this work (with the possible exception of TYMV) possess a central hole, of increasing size in viruses containing relatively less RNA: Within 10% the difference between the overall RNA radius (Table 3) and the radius of the hole is proportional to the molecular weight of the RNA in the virion.
- (2) The external diameter of the bulk of the RNA is much smaller than the virus diameter.
- (3) The degree of interpenetration of RNA and protein is different in TYMV and CMV. In TYMV there is little (if any) penetration of the RNA into the protein capsid (about 5 Å), whereas in CMV this penetration must be about 20 Å. This difference in penetration is due to a difference in the internal diameters of the capsids. Our model for TYMV is different from that of Klug *et al.*⁴, who suggested a strong imbedding of TYMV RNA into the capsid. The reason for this discrepancy will be discussed elsewhere¹⁶. We simply note here that our method gives in a fairly direct way the overall size of the RNA inside the virion, and that the high ionic strength used by Finch and Klug could induce a swelling of the RNA.
- (4) We found that in TYMV (and MS2), the maximum of the scattering density of the protein shell suggests a very dense packing, whereas for CMV the packing is less dense, leaving room for holes representing about 15% of the surface at the radius of higher density (117 Å). The presence of such holes could explain the sensitivity of CMV to ribonuclease, even if its RNA is localised in a sphere much smaller than the overall size of the virus. These differences between TYMV and CMV organisation could account for the difference between their properties. The isomorphism of TYMV and TYMV empty capsids, the high stability of these capsids and virions, and the insensitivity of TYMV to ribonuclease, can be well understood from the structure we found for the virion (Fig. 4). Conversely, the absence of empty capsids, the sensitivity to ribonuclease and to magnesium ions can be easily understood from the model we propose here for CMV.

MS2 seems similar to TYMV, in agreement with the known properties of that virus, in particular the possibility of obtaining empty capsids by alkaline treatment. Our data for SBMV suggest that this virus may have some features in common with CMV, in particular, the presence of holes in its capsid: its sensitivity to ribonuclease is suggested by other experiments²¹.

The case of BMV (ref. 25) is clearly intermediate between those of TYMV and CMV. In its low pH form it shows a small RNA penetration into the capsid (but larger than for TYMV or MS2). In its swollen, high pH form (which is likely to be an *in vitro* artefact), the RNA is strongly embedded between protein subunits. The fact that the BMV empty capsids, which can be formed at pH 4–5 (refs 22, 23), have a diameter intermediate between those of the two forms of the virion²⁴, suggests that both protein–protein and protein–RNA interactions contribute to the stability of that virus.

(5) If neutron low-angle scattering curves of virus solutions provide direct information on the protein–RNA interpenetration and on the density of the protein shell, very little can be said on the folding and the symmetry of the RNA at its contact with the protein shell. But it is clear that the RNA is, on average, loosely packed; the highest radial density measured corresponds to about 50% RNA, 50% solvent by volume.

In conclusion, neutron small-angle scattering of virus solutions allows a fairly clear correlation to be made for small RNA viruses between the packing of RNA and protein subunits, and the nature and importance of stabilisation forces. Deeper penetration of the RNA into the capsid seems to be correlated with the increasing importance of RNA–protein interactions. The technique, however cannot give data on the folding of the RNA. This could be achieved by neutron diffrac-

tion studies at low resolution of single crystals in various D₂O environments which will be undertaken soon.

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Collagen–mineral axial relationship in calcified turkey leg tendon by X-ray and neutron diffraction

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The axial relationship between the collagen and mineral components in calcified turkey leg tendon was investigated using low-angle X-ray and neutron diffraction. The results indicate that the mineral is arranged with the same axial periodicity as the collagen and occupies the gap region of the collagen structure.

MINERALISED connective tissue such as bone is a composite structure of protein, mineral and glycoproteins. The structural relationships between these components is still unclear although, individually, they are better understood.

In most hard tissues, the protein matrix is collagen which exists as long fibrils of diameter 200–1,000 Å and displays the 670 Å period of transverse bands seen in electron micrographs of normal tendon collagen^{1,2}.

The mineral is widely accepted as being calcium phosphate although there is disagreement as to the actual forms present. Crystalline hydroxyapatite seems to dominate³ but there is clear evidence that an amorphous phase is also present⁴. X-ray⁵ and electron microscope⁶ studies show that the crystals have their unit cells oriented in a constant manner to the collagen fibril axis, suggesting a close structural relationship between these two ordered components. It is widely agreed that the crystals have one long side oriented parallel to the collagen fibrils, and the length of this long side is quoted variously between 300–1,000 Å. It is also agreed that one lateral dimension of the crystals is about 40 Å, although it is not known whether they exist as rods or plates. Many accept

that the crystals are distributed along the collagen fibrils with the same 670 Å periodicity as collagen, but there is debate as to whether they are confined to the periphery or penetrate through the interior, and there is no certain knowledge about the axial location of the crystals with respect to the collagen period, although it has been suggested that they occur in the gap region⁸.

We are investigating these questions using low-angle X-ray and neutron diffraction of mineralised turkey leg tendon. This tissue was chosen because the collagen is highly ordered in the axial direction, therefore giving well oriented diffraction patterns. Also, the system has been well studied using electron microscopy⁹. Although not strictly bone, the tissue does nevertheless represent a simple collagen–mineral interactive system, the results from which can be usefully applied to the whole problem of calcified tissues.

Principles of the method

X-ray and neutron diffraction patterns from tendons contain, at low angles, a set of meridional reflections which index as orders of 670 Å (*D*), the periodicity of the collagen fibrils when projected on to the fibril axis. The *D* period arises because, in projection, the 2,990 Å (*L*) long collagen molecules are staggered axially by *D* with respect to each other (Fig. 1). Since *L* is approximately 4.5*D*, this molecular arrangement implies that each period has an overlap and gap region, each of approximately 0.5*D* in length.

The amino acid sequence of the collagen molecules is now known in some detail for rat and calf skin^{10,11}. It is therefore possible to use the sequence and the Hodge–Petruska scheme

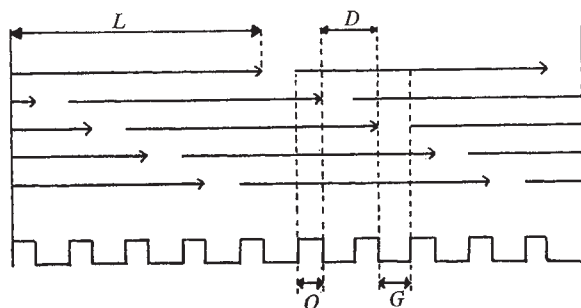


Fig. 1 Hodge-Petruska scheme for the molecular arrangement of collagen molecules¹⁹. L (2,990 Å) is the length of each collagen molecule and adjacent molecules are staggered by D (670 Å) with respect to each other. When projected on to the fibre axis, the arrangement produces a step function of period D containing an overlap region (O) and a gap region (G).

to construct the axially projected electron density (X-ray diffraction) and nuclear scattering density (neutron diffraction) profiles. From these models, the predicted intensities of the low angle X-ray and neutron meridional reflections may be calculated. It has been demonstrated^{12,13} that a good correspondence can be obtained between the calculated and observed intensities in both diffraction techniques. Uncalcified turkey leg tendons from young birds show a meridional pattern very similar to that of rat tail tendon, the major differences occurring at a resolution in the order of 20 Å. This represents a much higher resolution than will be described here (74 Å) and, therefore, the scattering density models obtained for the rat tail can be confidently applied to this study of the turkey tendon. Thus, by comparing the X-ray and neutron diffraction patterns of calcified and uncalcified tendon an attempt can be made to locate the mineral with respect to the collagen axial structure.

The major problem when trying to compare the diffracted intensities from two similarly periodic structures containing different amounts of scattering material is one of scaling. In this case, it was overcome (in both diffraction techniques) by measuring the relative first-order intensities of a sample before and after decalcification. Allowance was made for the different absorption properties of the specimen with and without the mineral by normalising the intensities to the measured trans-

mission of the undeviated beam. The important assumption that decalcified and uncalcified turkey tendon collagen are equivalent is substantiated by their X-ray diffraction patterns, which show no measureable differences to a resolution of 15 Å. Decalcification was carried out in phosphate-buffered EDTA (pH 8.0; 5%) followed by extensive washing in 0.15 M NaCl.

All experiments were carried out on leg tendons freshly dissected from turkeys killed in week 22 after birth. The tendons begin to calcify at week 16 and in the following 6 weeks calcify extensively to form stiff bone-like structures.

X-ray diffraction

Previous low-angle X-ray work¹⁴ on calcified systems, including the turkey leg tendon, has shown the existence of an axial repeat of spacing 660 Å, and the intensities of the first six orders have been quantified. It was observed that these reflections have a much higher absolute intensity than those from uncalcified tendon. Furthermore, the reflections were obtained with mineralised tendon from which the collagen had been removed. It was concluded that these reflections come from the mineral component distributed with the D period and not the collagen which exhibits a spacing of 640 Å in the dry state.

We have extended these observations. Figure 2 shows the low-angle meridional patterns from calcified and uncalcified turkey leg tendon as well as that from rat tail tendon for comparison. Table 1 lists the measured intensities of the nine orders which are seen in the diffraction patterns. Several important preliminary observations can be made.

As observed by other workers¹⁴, there is strong diffuse scatter from the mineralised tendon at low angles, which is confined to the equator. It is widely agreed that the origin of this scatter is the 'form factor' (or 'shape factor') of individual crystallites of mineral from which estimates of the crystal size can be made⁷.

The absolute intensities of the reflections are indeed much higher for the tendon in the calcified state which must be due to the extra scattering by the mineral, although orders 1–5 in each pattern have relative intensities which are very similar.

Finally, the spacings of the reflections from the calcified and uncalcified tissues are exactly the same (orders of 670 Å). Because the mineral absorbs the X rays to such a large extent (approximately 90% attenuation for the thickness of sample used, see Fig. 2) and scatters much more strongly than collagen, it could be argued that the calcified tissue reflections are due solely to the mineral arranged D periodically, and that the collagen reflections which may exhibit a different D periodicity

Fig. 2 Low-angle X-ray diffraction patterns showing the first nine meridional reflections of rat tail tendon collagen (a), uncalcified turkey tendon collagen (b), calcified turkey tendon collagen (c). A mirror-monochromator focusing system was used linked to a rotating anode source (Elliot Automation GX6) with a specimen-to-film distance of 85 cm. Thin specimens, approximately 0.2 mm in diameter were suspended over 0.15 M NaCl in a sealed Perspex cell with Mylar windows. The rat tail tendon and the uncalcified turkey tendon clearly give very similar diffraction patterns. d, Calcified turkey tendon, taken at the Synchrotron Radiation Facility (EMBL) at DESY, Hamburg, in collaboration with A. Harmsen (specimen-to-film distance was 39 cm). The higher orders of the collagen are just visible above the intense low-angle diffuse scatter from the mineral crystallites.

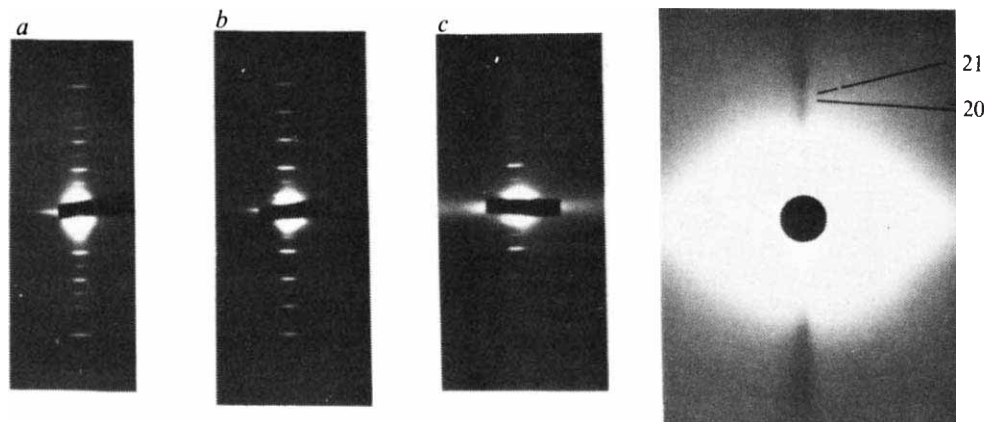


Table 1 Intensities of the first nine orders of the meridional X-ray diffraction patterns from rat tail tendon, uncalcified turkey tendon and calcified turkey tendon

Order	Rat tail tendon	Turkey tendon uncalcified	Turkey tendon calcified	Calcified collagen model
1	1,000	1,000	9,145	9,145
2	27	9	102	97
3	109	107	834	993
4	11	7	68	72
5	54	48	192	365
6	17	8	81	88
7	21	30	51	140
8	6	8	17	35
9	71	37	59	55

To measure intensities accurately, each diffraction pattern was digitised on an Optronics densitometer system, and the numerical data analysed with a computer program which integrates over each reflection and subtracts local background. Various corrections were applied to allow for the geometry of the X-ray camera, and other factors inherent in the measurement of fibre diffraction patterns. These are fully described elsewhere¹⁵. The arbitrary scale is 1,000 for the first order of uncalcified collagen. The final column lists the calculated intensities as obtained from the refined model of the calcified collagen.

are too weak to be seen. Very long exposures, however, fail to show up any other low-angle reflections. Furthermore, Fig. 2 also shows a diffraction pattern of mineralised turkey leg tendon obtained with Synchrotron Radiation at DESY in Hamburg. This very high-intensity X-ray source allows large specimen-film distances which reduces the diffuse scatter relative to the Bragg peaks whilst still retaining the ability to view the weak collagen reflections at higher angles. The higher orders of collagen, although still very weak, are clearly visible on the 670-Å spacing, especially the characteristically strong 20th and 21st.

Several important conclusions can be made. First, the mineral is arranged *D* periodically in the form of a step function along the collagen. The step width must be about $0.5D$ to give the strong, first, third and fifth orders. Second, the underlying collagen matrix retains its wet, native periodicity of 670 Å. At higher resolution, where the transform of the mineral component step quickly dies away, the collagen pattern can be seen.

The important question concerning the relationship between the two components, however, still remains unresolved. A specific interaction would mean that the amplitudes of the two individual transforms would add, whereas an incoherent arrangement would cause the intensities to add. The distinction cannot be readily made because the mineral scatter is so dominant. To help overcome this problem, the material was studied with neutrons.

Neutron diffraction

This is a particularly attractive technique to apply to calcified systems for two reasons. Unlike X rays, the nuclear scattering of neutrons by mineral and protein is comparable, and the former component does not dominate the resultant pattern. Second, we can use the fact that the background scattering by the water can be altered by introducing various amounts of D_2O into the system—the so-called contrast variation technique¹⁶. The average scattering length density of collagen at low resolution equals that of an aqueous background containing 47% D_2O , whereas that of the mineral is shown below to occur at a much higher value in the region of 80%. This large separation of 'matchpoints' between the two components means that we can effectively look at the scattering from one independently of the other. The behaviour of the rat tail collagen neutron diffraction pattern at different H_2O/D_2O ratios is well understood¹⁷ and can be used to interpret the results from the calcified collagenous system.

The first six orders from calcified turkey tendon were collected on the D-11 instrument¹⁸ at the Institut Laue-Langevin,

Grenoble in a range of D_2O-H_2O mixtures from 0 to 100% in steps of 20% (Table 2). Several important features of the diffraction pattern and its variation with percentage D_2O are clear.

At all contrasts, except that in 100% H_2O , the intensities indicate that the structure in projection has a step function nature in having relatively strong first and third orders and a weaker second. Nowhere, however, is it as pronounced as with the X-ray data, and this reflects the fact that with neutrons the mineral is not dominating the diffraction pattern.

If the intensities are converted to amplitudes (by taking the square root), it is possible to plot their variation with percentage D_2O for each order and in all cases the relationship is non-linear. For any centrosymmetric structure, it can be shown that the amplitudes of its Fourier transform will vary linearly with percentage D_2O (ref. 16) and this is true for the low resolution rat tail tendon data (unpublished results). Clearly, the calcified collagen structure in projection is not centrosymmetric.

The large increase in the sixth order is entirely consistent with the collagen component and essentially results from its amino acid sequence¹⁷.

Finally, the first-order intensity of calcified collagen is actually less than that of uncalcified collagen at 0% D_2O . If the intensities from each component were merely adding to produce the composite value, the first order for the calcified should be much higher than that of the uncalcified. The fact that this is not the case, strongly suggests that the amplitudes are adding with some phase relationship which in turn implies a specific interaction between the collagen and the mineral.

The results can be further analysed to give a low resolution picture of the relationship between the collagen and the mineral.

The X-ray results indicate that, at the resolution of the first order, both components can be considered as step functions. The first order of the composite diffraction pattern is a resultant of the two step function transforms at some phase angle *Q*. Therefore, determination of *Q* will give the axial relationship between each component. Contrast variation makes this possible using the neutron results.

Let A_M and A_C be the mineral and collagen first-order amplitudes in 100% H_2O , F_M and F_C the fractions of D_2O at which each amplitude falls to zero (the respective matchpoints), and A_{XM} and A_{XC} the amplitude values at a fraction *X* of D_2O . Each component, being a step function, is centrosymmetric and the amplitudes of their transforms will vary linearly with the D_2O composition. It follows that

$$A_{XM} = A_M - (A_M/F_M) X \quad (1)$$

$$A_{XC} = A_C - (A_C/F_C) X \quad (2)$$

Table 2 Variation of the first six orders of calcified turkey tendon meridional neutron diffraction pattern with percentage D_2O in the medium

Order	Calcified turkey tendon intensities % D ₂ O					
	0	20	40	60	80	100
1	861	481	349	357	659	979
2	25	13	10	15	30	42
3	29	29	17	30	84	139
4	4	3	5	11	20	26
5	10	8	2	4	13	21
6	4	6	13	31	61	85

Specimens of calcified tendon were prepared by laying slices of tendon side by side to form a sheet approximately 0.1 cm thick and 2 cm square. Large specimens are required to use fully the 1-cm² neutron beam. After thorough equilibration in the 0.15 M NaCl DO_2/H_2O mixture, the sheets were mounted in specially prepared sealed cells with quartz windows containing the same bathing medium. Six orders could be well resolved on the 64 × 64 cm² detector using a wavelength of 5.4 Å, and a specimen-detector distance of 5.53 m. As with the X-ray data, the numerical output from the detector was analysed by a program which calculates integrated intensities corrected for local background. All values are scaled relative to a first order value of 1,000 for rat tail tendon in 100% H_2O .

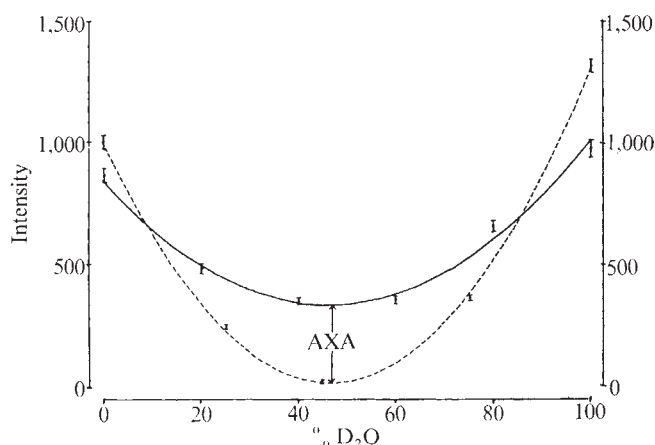


Fig. 3 Variation in first-order intensities of calcified turkey tendon (continuous line) and rat tail tendon (broken line) with percentage D_2O . The quadratic curves were fitted to the observed data using a least squares routine. The first cross-over point at 8% D_2O represents a point where the length of the two component amplitudes is such that their resultant is equal in length to the collagen amplitude alone. The second cross-over point at 86% D_2O represents the matchpoint of the mineral phase. At 47% D_2O where the collagen contribution is almost zero, one can estimate A_{XM} the mineral amplitude alone at this particular percentage D_2O .

Using the cosine rule, the resultant intensity variation of the composite first order with D_2O is

$$A_{MCX}^2 = X^2((A_c^2/F_c^2) + (A_m^2/F_m^2) + (2A_cA_m/F_cF_m)\cos Q) - X((2A_c^2/F_c) + (2A_m^2/F_m) + (2A_cA_m/F_m)\cos Q) + (2A_cA_m/F_c)\cos Q + (A_c^2 + A_m^2 + 2A_cA_m\cos Q) \quad (3)$$

This equation predicts a quadratic variation in the first-order intensity with respect to fractional D_2O , and Fig. 3 shows a quadratic curve fitted to the results together with the rat tail tendon first-order intensity variation. The cross-over point provides a value for the mineral matchpoint F_m (0.86 D_2O). At 0.47 D_2O , the intensity of calcified system gives an estimate of A_{XM} (18.3) where the collagen contribution is at a minimum, and therefore A_m can be calculated from equation (1) as 40.4. A_c and F_c (31.42 and 0.47 D_2O) are known from the rat tail tendon data (unpublished results). Using equation (3), a value for Q can be found which gives maximum agreement with the quadratic curve fitted to the data shown in Fig. 3. The value resulting from such an analysis is 134° .

Refinement

Can this very crude model of two-step functions related by a phase angle of 134° be improved? The nine orders of the X-ray pattern of calcified tendon provides higher resolution information than the six orders observed using neutrons, and these can be used to refine the structure. Although the first few orders are dominated by the mineral, this is not true of the higher orders where the mineral transform is quickly dying away. The model structure for rat tail tendon collagen provides a reliable source of phases of the first nine orders of the X-ray pattern which can be combined with observed intensities to give a low resolution model of the uncalcified tendon. By adding a step function to represent the mineral, it is possible to calculate the expected X-ray intensities from the composite system and compare them with the observed. The length of the step function was varied between $0.45D$ and $0.55D$ (as indicated by the X-ray results), and its position varied between $120^\circ \rightarrow 170^\circ$ and $-120^\circ \rightarrow -170^\circ$ relative to the collagen step function (as indicated by the neutron results). Both angular regions need to be included because the centrosymmetric assumption used in the neutron diffraction data analysis cannot distinguish between these two possibilities. For convenience, observed and calculated first-

order intensities were equalised by adjusting the height of the mineral block, and an R factor $\Sigma(|F_{obs}| - |F_{calc}|)/\Sigma|F_{obs}|$ was calculated on the amplitudes of the other eight orders to provide a measure of agreement.

The average R factor obtained within the refinement region was 0.49, but with a step width of $0.48D$ and a phase shift of $+160^\circ$, a minimum value of 0.17 was obtained. This minimum is 2.8 s.d. units from the mean. For structures chosen randomly outside the refinement region nowhere was a value less than 0.6 observed. The calculated intensities from the best fit model are listed in Table 1 for comparison with the observed data.

Figure 4 is a Fourier synthesis of the composite structure using the model phases and observed X-ray amplitudes.

Discussion

The axially projected structure proposed for the calcified turkey tendon shown in Fig. 4 is based primarily on the results of X-ray diffraction, but it is consistent with the neutron diffraction. The observation that the first-order intensity with neutrons in 100% H_2O is higher for the uncalcified than for the calcified tendon is consistent with the component step functions being 160° out of phase, and the neutron scattering from each being comparable. In effect, the mineral scatter is cancelling that of the collagen and, therefore, removal of the former increases the first-order intensity. This also explains why the step function is enhanced at higher percentages of D_2O in the medium. In the middle range near the collagen matchpoint (30–60%) where its contribution is small, the mineral step dominates; the reverse is true in the higher range (70–100%) near the mineral matchpoint.

As predicted, the structure is not centrosymmetric, which would only be the case if the phase angle between the two first order components were close to 0° or 180° . The disparity between the phase angles obtained from the two diffraction techniques may seem rather large. The difference of 26° only represents, however, an axial distance of $48 \text{ \AA}$ which is less than the nine-order ($74 \text{ \AA}$) resolution of the model, and the value of 134° obtained from the neutron data analysis did provide a useful starting point for the refinement using the higher resolution X-ray data.

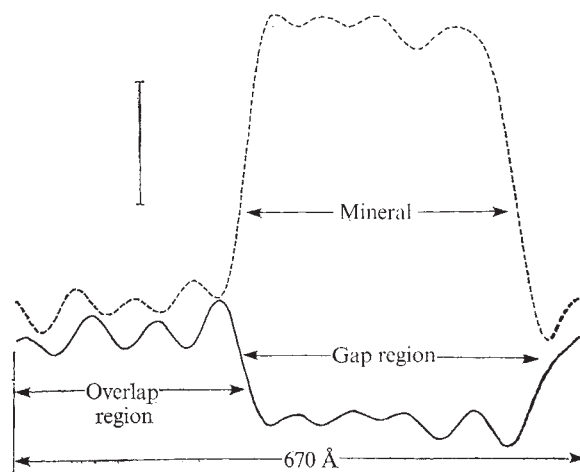


Fig. 4 Fourier syntheses of the uncalcified (continuous line) and calcified (broken line) turkey tendon with a D period. Both were constructed using the observed intensities and calculated phases of the first nine orders—the uncalcified collagen phases from the rat tail tendon model, and the calcified collagen phases from the refined model described in the text. For clarity, the two curves are slightly offset, but the overlap region of the uncalcified collagen corresponds in electron density to the region of the calcified profile where there is no mineral contribution. Exact axial register between the minor peaks on each curve is not to be expected at this poor resolution where distortion due to termination errors is very high. It is clear, however, that the mineral is lying in precise axial alignment with the gap region. The vertical bar represents a height of 50 electrons per residue translation.

This work has shown that a definite axial relationship exists between mineral and collagen in calcified turkey leg tendon. The most attractive feature of the model is that the size of the mineral block and its position places it precisely in the gap region. Although these results provide no information concerning the lateral arrangement of the two components, it is difficult not to speculate that the mineral does penetrate the fibril and occupy the gap region. As indicated by others⁸, this is the only way that penetration of the collagen by the mineral could occur without disrupting its fibrillar structure. The height and length of the artificial step, used to represent the inorganic phase in the model, corresponds to an actual volume of about 30,000 Å³ per collagen molecule (using the apatite electron density). Taking the mineral block length as 0.48D (320 Å), this would correspond to a cross-sectional area of 94 Å². This may be considered rather low when compared with the crystal size estimates mentioned earlier, but it must be stressed that the tendons from 22-week-old birds are certainly not fully calcified, and that this value of 30,000 Å³ only represents some kind of average over the tendon. A quantitative chemical analysis of the amount of mineral present in the tendons of birds at various ages should help to clarify this point.

Preliminary X-ray pictures of bat phalangeal bone indicate a similar low-angle diffraction pattern to that of calcified turkey tendon, and it is hoped that work on this and similarly well oriented hard tissues will bridge the gap between the calcified tendon system and true bone.

The initial X-ray work described was carried out in the Laboratory of Molecular Biophysics, University of Oxford, and we thank Professor D. C. Phillips, Dr C. Rodger and Dr B. Brodsky (Doyle) for their advice and assistance during this period. We thank the Arthritis and Rheumatism Council and the European Molecular Biology Organisation for support, and the ILL for neutron scattering and computing facilities.

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letters to nature

The possible galactic origin of γ -ray bursts

FOLLOWING the discovery of non-solar γ -ray bursts¹, various models to account for their origin have been discussed. Extragalactic models include supernova bursts in remote galaxies², and the collapse of the cores in active stars³, while galactic models are based on flare stars⁴, thermonuclear explosions on neutron stars⁵ and the sudden accretion of cometary gas on to neutron stars⁶. The acceptability of any of these models may be tested by the observed size spectrum of γ -ray bursts. The extragalactic models predict a power law spectrum with $\alpha = -1.5$ while for the galactic models the number index will be -1 . The experimental data on γ -ray bursts is still meagre. So far, about 44 confirmed events have been recorded by satellite-borne instruments^{7,8}. The number spectrum of the observed γ -ray bursts (Fig. 1) shows that the observed distribution for events with an energy less than 10^{-4} erg cm⁻² is flat and thus makes the choice of any model completely arbitrary. Carter *et al.*⁹ presented an upper limit on the number of γ -ray bursts having an energy $> 2 \cdot 10^{-7}$ erg cm⁻² and suggested that the data are consistent with a power law spectrum in which $\alpha \sim 0.5$, or a modified galactic distribution. We present here an alternative analysis of the observed γ -ray events, which suggests very interesting possibilities for their origin.

In Table 1 we have listed the chronological catalogue of the observed γ -ray events. Inspection of the data relating to the estimated flux suggests that there is a preferred mean energy for γ -ray bursts. Some 90% of the events have an energy between 5×10^{-5} and 5×10^{-4} erg cm⁻², contrary to the predicted characteristics of the number spectrum of various models even if a detection bias of 10 is taken into account for low energy bursts.

We estimate in this analysis the relative distance of the origin of each burst by assuming a constant intrinsic luminosity of the source. The data were then divided into 10 bins. The number of bins is chosen to reflect the observed variations in energy by two orders of magnitude. In the histogram obtained from this analysis (Fig. 2a), the numbers are plotted using an arbitrary distance scale. In Fig. 2b we have plotted the galactic distribution of HII regions and clouds of molecular hydrogen¹¹. The remarkable similarity between the distribution of γ -ray bursts, and that of supernova remnants, suggests a genetic relationship between the two and the galactic origin of the γ -ray bursts. A selection bias in binning the data to generate the γ -ray burst histogram can alter the shape slightly but the overall gaussian nature is preserved under all combinations.

If the apparent correlation shown in Fig. 2a between γ -ray bursts and supernova remnants is genuine, then the burst source could be identified with 'cool', that is, completely run down neutron stars. Current theories of supernovae suggest that each supernova explosion results in the formation of a neutron star. This prediction has been further supported by the discovery of pulsars. About 20% of these compact objects will be in close binary systems. Most of these neutron stars will lose their angular momentum in 10^4 – 10^6 yr, because $\Omega/\dot{\Omega} = P/\dot{P} \sim 10^{-12}$ s, is representative of the observed values of P in various pulsars. Thus, at any time, there exists a large population of 'cool' neutron stars in our Galaxy. We propose that γ -ray bursts originate from the disruption of these 'cool' neutron stars.

Lattimar and Schramm¹² investigated a mechanism whereby a neutron star falling towards a black hole is tidally disrupted, for example, in a binary system. This results in the decompression of the ejected neutron-star matter. As this material expands it follows a unique evolutionary path, like the r-process,

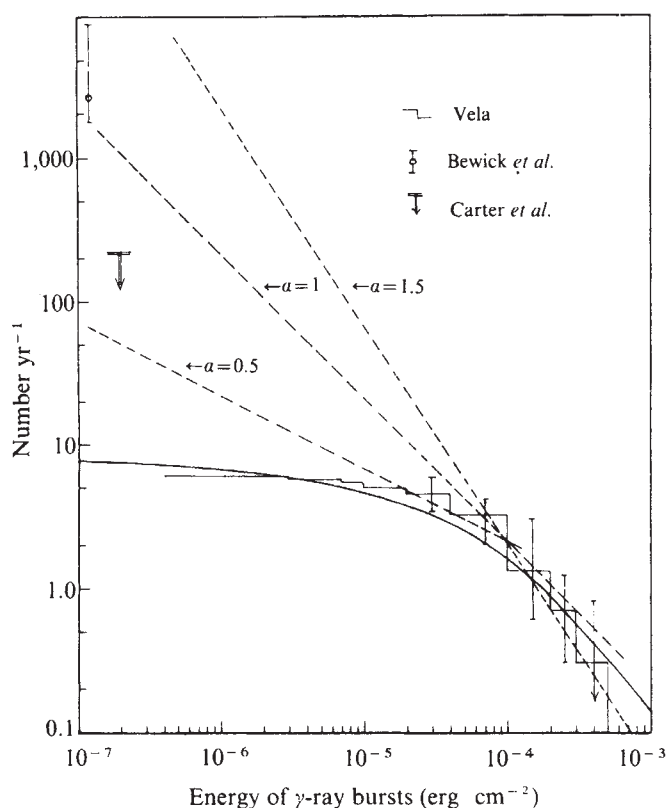


Fig. 1 The number spectrum of the observed γ -ray bursts. The three dotted lines labelled $\alpha = 1.5, 1$ and 0.5 represent the power law function $N \propto S^{-\alpha}$, predicted for sources distributed uniformly in 3, 2 and 1 dimensions, respectively. —, Cumulative gaussian distribution normalised at $5 \times 10^{-4} \text{ erg cm}^{-2}$.

towards the region of superheavy elements¹³, and nuclei up to mass $A \sim 300$ are formed. Fission then sets in until such time that all the neutrons are used up. This process would result in a γ flash. The characteristic time scale for such a process is from 10 ms to a few seconds. If we take account of the comparatively long decay times of the various isotopes and the β -decay life time of the various intermediate nuclei, the observed time structure of γ -ray bursts can easily be accounted for. It is interesting, however, that some of the observed features will follow naturally from such a model.

- (1) The spectral shape for all events will be similar. This is suggested by the spectra of the 14 events observed by the IMP satellite⁸.
- (2) The energy released during each disruption will be similar, as there is only a small range in the permitted mass of neutron stars.
- (3) The expected rate of occurrence of γ -ray bursts in the entire energy range should remain constant within statistical limits.

The present model can also provide the required energy, which at a distance of 6 kpc and an average observed energy of $5 \times 10^{-4} \text{ erg cm}^{-2}$, corresponds to $10^{42} \text{ erg s}^{-1}$ emitted in γ rays during the burst. Assuming that 1% of the total energy is radiated as γ rays, the total energy released corresponds to $10^{44} \text{ erg s}^{-1}$. This value is well within the range of the energies associated with stellar explosions. As the energy is released in the disruption of the core, the restrictions observed in accreting models due to the Eddington limit ($\sim 10^{39} \text{ erg}$ for the case of a neutron star having a mass of $1 M_{\odot}$) do not apply.

It is possible to predict the form of a number spectrum of the observed events if the association with supernova remnants is valid and neutron star disruption results in γ -ray bursts. The

number spectrum predicted by such a model can be defined as a product of three distributions.

$$N(E) = N_C(t)N_L(E)N_R(r)$$

where N_C is the number distribution for star disruption, N_L the distribution of the luminosities of burst sources, and N_R the distribution of sources with distance.

All three distributions in the equation can be approximated by gaussian functions. The first two represent statistical phenomena, and the observed distribution of N_R seems to be gaussian as is indicated in Fig. 2a. Therefore, the observed composite distribution will also be gaussian. The integral number spectrum of burst events per yr will then be represented by a cumulative gaussian function. Figure 1 shows that such a distribution fits the observed number distribution extremely well.

The present model predicts an anisotropy in the arrival directions of the bursts. The magnitude of this anisotropy will depend on the spatial distribution of the 'cool' neutron stars. Any analysis of this nature is limited by the very small number of observed burst (50) and the fact that the arrival directions

Table 1 Chronological catalogue of γ -ray events

Serial No.	Date	Time (UT)	Estimated flux (Xfu)*	
			Vela†	Imp‡
67-1	2 July 1967	14 19 28	100	
69-1	3 July 1969	07 17 13	20	
69-2	19 July 1969	03 46 46	100	
69-3	7 October 1969	07 26 31	200	
69-4	17 October 1969	03 18 47	20	
69-5	17 October 1969	21 41 53	40	
70-1	25 January 1969	05 01 30	—	
70-2	10 July 1970	05 17 46	40	
70-3	22 August 1970	16 49 31	100	
70-4	1 October 1970	15 42 16	100	
70-5	1 December 1970	20 00 59	4	
70-6	30 December 1970	07 02 17	300	
71-1	2 January 1971	19 10 56	100	
71-2	27 February 1971	17 27 35	70	
71-3	15 March 1971	11 20 27	50	
71-4	18 March 1971	15 28 05	100	
71-5	21 April 1971	03 18 39	3	
71-6	30 June 1971	17 30 59	500	
72-1	17 January 1972	17 39 16	70	
72-2	12 March 1972	15 53 15	50	
72-3	28 March 1972	13 46 28	100	
72-4	27 April 1972	10 58 32	30	
72-5	14 May 1972	03 46 31	200	
72-6	1 November 1972	18 56 46	7	
72-7	13 November 1972	16 02 38	10	41
72-8	18 December 1972	20 27 39	100	120
73-1	25 January 1973	15 14 57	3	46
73-2	2 March 1973	23 27 58	300	280
73-3	16 April 1973	12 38 57	30	30
73-4	7 May 1973	08 04 32	6	—
73-5	17 May 1973	01 34 38	—	36
73-6	6 June 1973	07 07 28	100	60
73-7	6 June 1973	18 47 14	—	71
73-8	10 June 1973	20 50 42	100	90
73-9	21 July 1973	08 55 18	200	120
73-10	25 July 1973	17 07 57	200	65
73-11	20 August 1973	16 39 16	—	27
73-12	18 September 1973	05 11 00	200	—
73-13	26 September 1973	16 04 31	30	70
73-14	17 December 1973	08 09 08	80	110
73-15	23 December 1973	00 39 47	80	62
74-1	21 January 1974	18 05 22	—	42
74-2	2 April 1974	18 20 38	—	41
74-3	19 April 1975§	17 14 35	—	30
74-4	28 July 1974§	14 01 46	—	27
74-5	6 August 1974§	10 36 37	—	420
74-6	23 August 1974	14 35 44	—	20
74-7	29 September 1974	18 51 45	300	—
74-8	14 December 1974§	10 14 53	—	—

*1 Xfu (X-ray flux unit) = $10^{-6} \text{ erg cm}^{-2}$.

†Energy range 150–750 keV (Vela 5) and 300–1,500 keV (Vela 6).

‡Measurements between 100 and 1,000 keV.

§Unconfirmed.

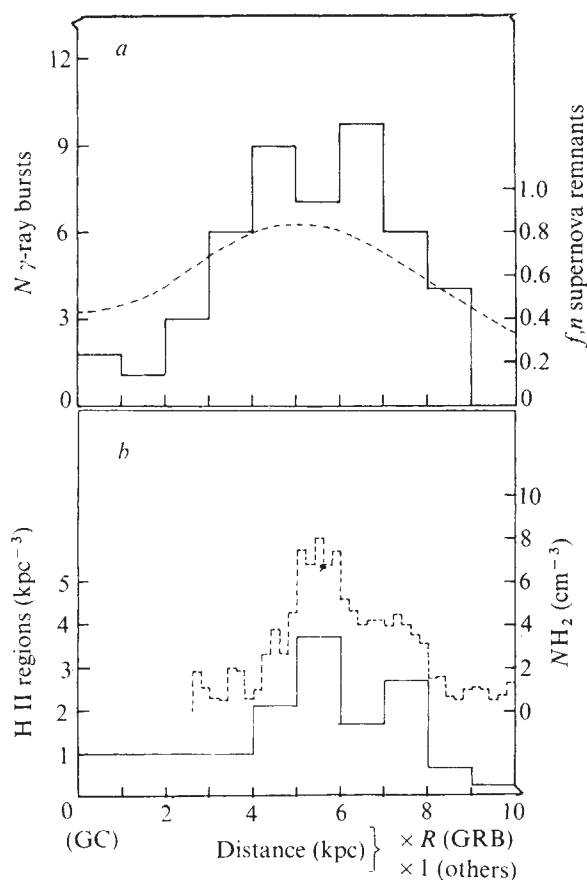


Fig. 2 *a*, The distance distribution of γ -ray bursts (—, arbitrary scale with galactic centre (GC) at 10 kpc) is plotted along with the inferred galactic distribution of supernova remnants (---) ¹⁰. *b*, Radial distribution of other galactic constituents, H II regions (—) and the molecular hydrogen (---).

have large error boxes, except for a few cases. As most of the events were seen by the Vela satellites, the inferred positions have relatively larger errors in galactic longitude than in galactic latitude. Strong *et al.*¹¹ have analysed the latitude dependence of 16 γ -ray bursts. The resulting distribution deviates significantly from that expected on the basis of isotropic distribution, suggesting that γ -ray burst sources indeed cluster near the galactic equator. This conclusion is based on a small sample of observations, and therefore we do not yet regard this as firm supporting evidence.

The present apparent isotropic nature of γ -ray burst sources may be misleading due to poor statistics. More directional data are required to establish the form of the frequency distribution.

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Possible detection of a radio event correlated with a γ -ray burst

COSMIC bursts of electromagnetic radiation, both isolated and in connection with other impulsive astrophysical phenomena, have been sought for many years¹, with only one suggestive positive result², until, in 1973, the discovery of γ -ray bursts³ of cosmic origin prompted a new series of observations⁴. In 1975 we started a systematic search for electromagnetic bursts at v.h.f. and u.h.f. The system, based at Medicina (Bologna, Italy), is semi-automatic and assures highly efficient data collection. Simultaneous observations at different frequencies facilitate the evaluation of disturbances of local origin and, hopefully, their isolation. Regular observations started in July 1976, and we report here our detection of a radio burst, which seems to be correlated with a γ -ray burst.

On August 16 1976, a radio burst was detected at Medicina at four different frequencies⁵. About 60 s earlier, three satellites (1 Vela, 2 Solrads) (R. W. Klebesadel, personal communication) and one balloon-borne detector on its second transatlantic flight (M. Sommer, personal communication) detected an intense γ -ray burst.

Details of the radio and γ data are given in Table 1.

The 151- and 408-MHz signals were detected by north-south arrays of 10 dipoles (HPBW = $15^\circ \times 75^\circ$ and $12^\circ \times 75^\circ$, respectively), used as transit instruments pointing along the meridian at an altitude of 16.5° . The 323.5- and 330.5-MHz data were obtained using two receivers connected to a 7° HPBW parabola (10-m diameter) tracking the Sun. The parabola operated in connection with the X-ray detector on its second transatlantic balloon flight. From the recordings (Fig. 1) it seems that the pulses have a duration of 30 s, which is long compared with the response recovering time of the pen recorders. On the same charts are several interference spikes whose width, much narrower than this, results from the finite thickness of the pen trace.

The similar duration and coarse structure of the radio pulses, evident at all frequencies, the coincidence within the resolution time between the signals detected by the arrays and the Medicina parabola (which are 700 m apart from each other), and the detection at four different frequencies make a man-made origin of the signal very improbable.

Strong evidence of a non-local origin of the phenomenon comes from the independent observation carried out at the Osservatorio Astronomico di Trieste (400 km away) (Table 1). At 237 MHz, with a 15° HPBW parabola (10-m diameter) monitoring the Sun, a structured event above the noise appears on the record in coincidence with our radio burst.

An atmospheric origin seems unlikely, first because the weather was clear and fine when the burst was detected, and second, because of the time coincident event at Trieste. From the rate of isolated pulses seen in coincidence within 10 s by the parabola and the arrays at Medicina, we expect a rate of four-fold random coincidence of 1 per 24 h. If we consider the Trieste dish, the rate of fivefold random coincidences drops to 1 per 432 h.

The γ -ray event occurs ~ 60 s earlier. Assuming a coincidence time of 2×60 s, the probability of a random correlation between the γ burst and the fivefold radio coincidence is 8×10^{-5} . This supports the hypothesis that the radio signals are the counterpart of the γ event, although a definite correlation can be given only when the location of the γ and radio event is found. To

Table 1 Radio and γ data for August 16 1976

Detector type	Array of dipoles		Medicina parabola		Trieste parabola	Balloon X-ray telescope
Frequency (MHz)	151	408	323.5	330.5	237	—
Bandwidth (MHz)	1	2	2	2	2	—
Energy Range (keV)	—	—	—	—	—	50–250
Onset time (UT in h, min, s)	16.16.24 (± 5 s)	16.16.24 (± 5 s)	16.16.25 (± 3 s)	16.16.25 (± 3 s)	16.16.20 (± 20 s)	16.15.27
Duration (s)	30	30	30	30	40	30
Integration time (s)	0.3	0.3	1	1	0.5	—
Peak intensity ($\text{W m}^{-2} \text{ Hz}^{-1}$)	8×10^{-22}	9×10^{-22}	3×10^{-23}	1.4×10^{-23}	2×10^{-22}	—

pinpoint the position of the radio burst source in the sky we could in principle convolve the heights of the signals measured at the various frequencies with the aerial beam patterns.

When the event occurred, the axes of the parabola and of the arrays were roughly orthogonal. The relative peak intensities of the pulses cannot be reconciled with the source on the axis of any of the aerials. The Sun is then excluded, being on the axis of the two parabola. A non-solar origin of the radio event is also supported by the available information on the Sun: on August 16 there was no evidence of activity in H (ref. 6), while at radio frequencies only a weak solar storm, not polarised, was present. Furthermore, a purely phenomenological analysis of the signals seen at the five different frequencies excludes the hypothesis of a typical solar radio burst.

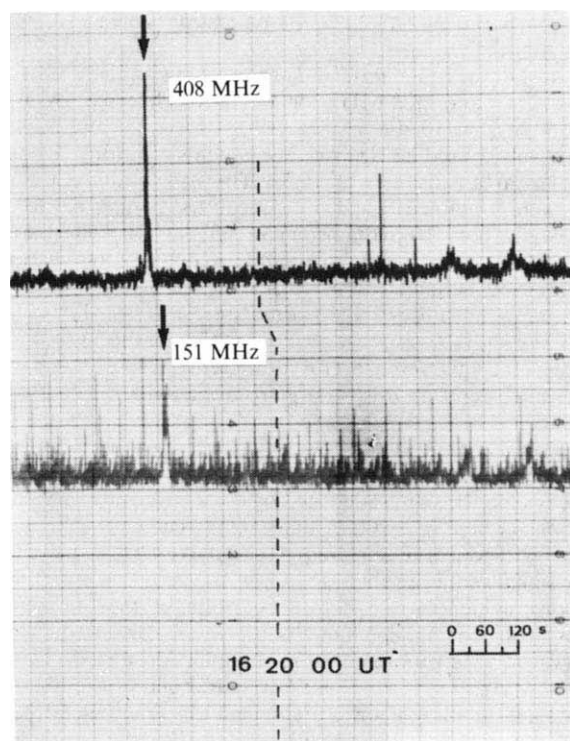


Fig. 1 Signals recorded on August 16 at 151 and 408 MHz. (Chart speed 60 cm h⁻¹.)

The decreasing peak intensities of the signal detected at 237, 323.5 and 330.5 MHz, respectively, and the frequency dependence of the parabola beam shape suggest that the source was at least 8° off the Sun position.

Analysis of the general trend of the power patterns of the aerials and their relative positions shows that the beam overlaps were only marginal. Here, the influence of side lobes is dominant

and difficult to measure. Precise calculations of the beam pattern intersections are therefore useless and probably misleading. Hence we limit ourselves to defining a region of sky (shaded area in Fig. 2) where the radio burst most probably originated. Some interesting objects exist in this region. Its area is so large, however, that any attempt to identify a precise object is meaningless.

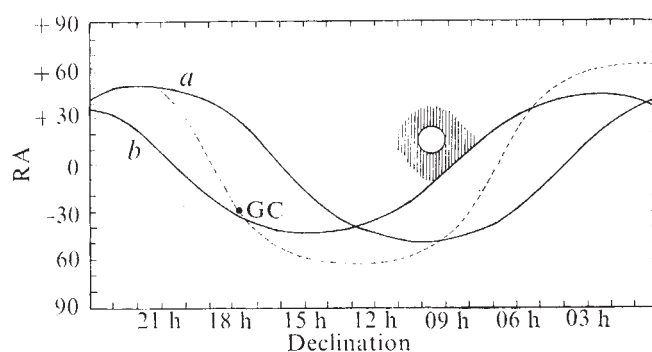


Fig. 2 *a*, Medicina horizon; *b*, balloon horizons, in equatorial coordinates. The radio burst location is marked by the shaded area. — —, Galactic equator; GC, Galactic centre.

We notice that all the possible positions of the source are at least 15° above the galactic plane and a delay between the 151- and 408-MHz radio signals produced by interstellar dispersion not greater than 10 s must be expected. It agrees with the upper limit we can assign to the delay between the extreme frequencies used in our experiment. The 1-min time difference between the onsets of γ and radio signals cannot be produced by dispersion. The γ -radio delay might then reflect the time evolution of the phenomenon which produced the electromagnetic burst.

Also, the duration δt of the radio burst, even in the case of the lowest frequency 151 MHz, cannot be attributed to dispersion across the bandwidth of the receiver. In fact, if the full 60-s delay between the γ -ray burst and a truly prompt radio burst were caused entirely by electron dispersion, δt would be only 0.8 s for a bandwidth $\Delta\nu = 1$ MHz.

Our radio data seem to be the radio counterpart of August 16, 1976, γ -ray burst. The probability of a random coincidence (8×10^{-5}) could have been drastically reduced by other independent observations. Unfortunately, this was not the case. We hope that more extended collaborations between various spaced stations will increase the efficiency in rejecting interference events and give further evidence of the existence of radio bursts of cosmic origin.

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Observational test for the existence of a rotating black hole in Cyg X-1

X-RAY satellite experiments^{1,13,14} are now investigating the degree and plane of linear polarisation of the radiation from Cyg X-1. This radiation can be explained as coming from an accretion disk around a black hole, the polarisation of the X rays being due to electron scattering in the hotter, inner regions of the disk. Novick reported² observing 3% linear polarisation (marginally significant at the 2.5 σ level) at 2.6 keV from Cyg X-1. Existing predictions of the polarisation properties, as a function of energy, have been based on a Newtonian approximation³⁻⁵, thus neglecting gravitational effects on the rays as they propagate from the disk's surface to an observer at infinity. We present here preliminary results of a full general-relativistic calculation which show that gravitational effects completely alter the polarisation properties, and provide a sensitive test of the existence of a black hole.

Whereas in the Newtonian approximation the plane of polarisation as measured at infinity is the same for each point of origin on the disk (for the same initial polarisation), it varies considerably when we allow for general-relativistic effects. These rotational differences can amount to over 100°. Even the special-relativistic aberration contributions, in the regions of interest, are typically an order of magnitude smaller than those of a gravitational origin.

We use the general-relativistic parallel-transport equations⁶ to propagate the polarisation vector from each point on the disk along the null geodesic linking it to an observer at infinity with polar angle θ_0 . While a non-rotating, Schwarzschild, black hole can be treated analytically (R. F. Stark, unpublished work and ref. 7), we report here numerical results for a Kerr black hole. For a rapidly rotating black hole the general-relativistic effects on the polarisation properties are an order of magnitude greater than for a slowly-rotating black hole or for a neutron star.

The degree of linear polarisation, δ , of the rays as they leave the disk will also differ from the Newtonian value. Gravitational bending of the light will alter the angle at which a ray leaves the disk's surface from the Newtonian value of θ_0 . As δ depends on the initial direction of travel (varying, for an optically thick disk, from 0% for rays leaving the disk vertically, up to 12% for rays leaving parallel to the disk)⁹, we can also expect gravitational effects to change the observed degree of polarisation.

We sum contributions from all parts of the disk to find the net observed polarisation properties, as a function of observed X-ray energy E_0 , from the net Stokes parameters $\bar{I}(E_0)$, $\bar{Q}(E_0)$, $\bar{U}(E_0)$, $\bar{V}(E_0)$ (ref. 8). Following Cunningham⁹, we use a transfer function f to obtain the intensity, but also define a polarisation transfer function $h = fe^{2i\phi}$ where ϕ is the observed angle of the plane of polarisation (measured from the unrotated direction). The net Stokes parameters can then be expressed as

$$\bar{I}(E_0) = \iint G(g) f I(E_0/g) r dr dg$$

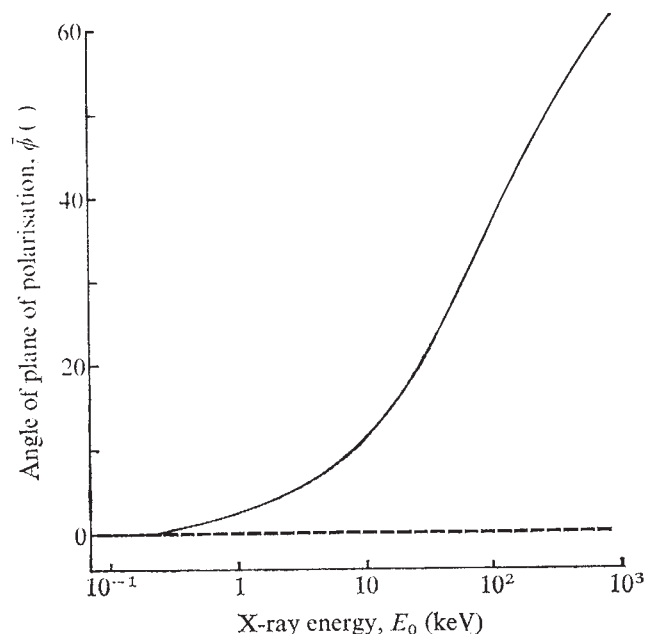
$$\bar{Q}(E_0) + i\bar{U}(E_0) = \iint G(g) h \delta(E_0/g) I(E_0/g) r dr dg$$

where G is a function only of the redshift g , and I is the locally produced intensity. The assumption of a plane, homogeneous atmosphere for the disk implies that $\bar{V}(E_0) = 0$, that is, there is no circular polarisation. The net degree of polarisation, and net polarisation angle, $\bar{\delta}(E_0)$, $\bar{\phi}(E_0)$, as observed at infinity are then given by:

$$\bar{\delta}(E_0) = \sqrt{[\bar{Q}^2(E_0) + \bar{U}^2(E_0)]/\bar{I}(E_0)}, \tan 2\bar{\phi} = \bar{U}(E_0)/\bar{Q}(E_0)$$

For illustration, we have used the Novikov-Thorne co-rotating, equatorial disk model¹⁰, for a Kerr black hole with specific angular momentum $a/m = 0.9981$ (ref. 11) although, as we emphasise, and discuss below, we expect similar effects with most disk models centred on rapidly rotating black holes. For our chosen parameters the disk could always be considered to be optically thick throughout. We use Chandrasekhar's results⁸ for the variation of δ with angle for such an atmosphere,

Fig. 1 Variation of plane of polarisation with energy for $\theta_0 = 75.5^\circ$. The parameters used for the disk model were: viscosity parameter, $\alpha = 0.1$; mass of black hole, $M = 9M_\odot$; mass accretion rate $= 7 \times 10^{17} \text{ g s}^{-1}$. —, General-relativistic curve; ---, Newtonian curve.



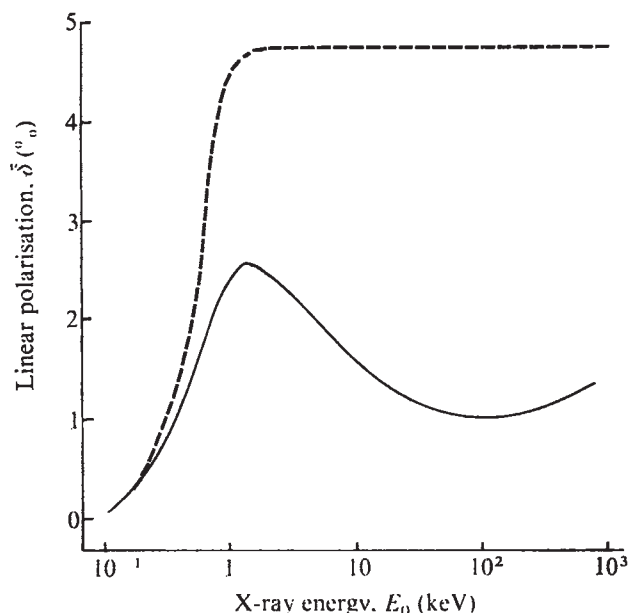


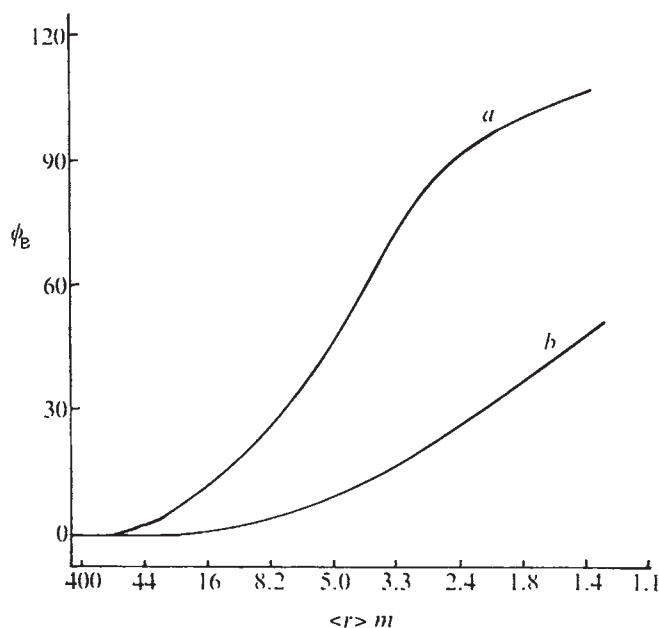
Fig. 2 Variation of linear polarisation with energy. Same parameters as in Fig. 1. —, General relativistic curve; ---, Newtonian curve.

modified to include frequency-dependent effects due to free-free opacity.

Figure 1 shows the large general-relativistic variation of the polarisation plane with energy. An anti-clockwise rotation with increasing energy will be seen by an observer above the disk (that is, with $\theta_0 < 90^\circ$), while observers below the disk will see a clockwise rotation. The effect of general relativity on the degree of polarisation (Fig. 2) is to cause a peak (below the predicted Newtonian value) and a fairly rapid fall-off at ~ 2 keV, and to cause a minimum and subsequent rise at higher energies. This behaviour is predominantly due to the gravitational bending of the rays previously mentioned, the depolarisation due to differential rotations in ϕ being smaller.

A large general-relativistic rotation in the plane of polarisation can be expected for most other disk models incorporating

Fig. 3 Variation of the polarisation plane of the most blueshifted radiation with emission radius. $a/m = 0.9981$. a, $\theta_0 = 41.1^\circ$; b, $\theta_0 = 75.5^\circ$.



electron scattering, and a good estimate of the rotation for these models can be found by examining the peaking behaviour of the integrals with redshift and radius. For a chosen model, as E_0 increases, I peaks for an annulus of decreasing radius, on account of the radial temperature structure of the disk. Around this annulus the redshift factor G peaks where the rays leaving it are blueshifted the most into E_0 . We thus find that the main contribution to the integrals comes from a small area of the disk. Figure 3 shows the variation of the observed polarisation plane ϕ_B of these maximum blueshifted rays with annular radius $\langle r \rangle$.

Once the variation of $\langle r \rangle$ with E_0 for a particular model has been found, the variation of ϕ_B with $\langle r \rangle$ can be used to obtain a good estimate for ϕ . If the disk has an optically thin inner region, $\bar{\phi}$ will change by 90° as $\langle r \rangle$ passes through the transition region from thick to thin¹², and will then resume a general-relativistic variation. Large variations in $\bar{\phi}$ with energy, of a gravitational origin, will occur whether the inner region is optically thick or thin (as in the two-temperature disk model)¹⁵.

The very large general-relativistic rotations in the plane of polarisation provide an opportunity for testing the black hole hypothesis for Cyg X-1. To observe these effects, X-ray satellite experiments are required with more sensitive polarimetry across a wider energy range than is available at present. The results also indicate that the polarisation properties of radiation emitted from regions of strong gravitational fields can be both theoretically and experimentally important.

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X rays from radio binaries

THE properties of the radio binary systems, CC Cas, AR Lac, β Per (Algol), β Lyr, b Per and Cyg X-1 have been listed¹. A thermal interpretation of the radiation from Algol² requires a much larger X-ray flux than the observed value of 3.8×10^{11} erg cm⁻² s⁻¹ keV⁻¹ in the 2–6-keV energy range³. The observation of some non-thermal flares, and the small size of the radio source in Algol indicate the non-thermal nature of the radio emission¹. We interpret the radio emission as synchrotron radiation and suggest that the observed X-ray emission is inverse Compton scattering of the light of the primary star by the radio electrons. We calculate X-ray emission from other radio binaries using this model. The energy for the radio electrons can arise from annihilation of magnetic lines connecting the binary stars twisted by the rotation of the stars⁴.

The energy of the synchrotron electrons of β Per (Algol) is fixed by the optical photon energy (about 0.8 eV) and the X-ray photon energy (4 keV); the relativistic factor for these electrons is about 75. The observed radio frequency (about 8×10^9 Hz) gives the strength of the magnetic field as 1.6 G in the radio-emitting region, using the usual synchrotron radiation formulae. The radio emission from the electrons, with the observed radio energy emission¹, gives the number of radio electrons as 2×10^{38} . The optical luminosity of

TABLE 1 X-ray flux from radio binaries

	CC Cas	AR Lac	β Per	β Lyr	b Per	Cyg X-1
Optical luminosity (erg s ⁻¹)	5×10^{36}	2×10^{34}	3×10^{35}	6×10^{36}	1.2×10^{35}	5×10^{36}
Maximum radio luminosity at 8085 MHz (erg s ⁻¹ MHz ⁻¹)	1.2×10^{19}	7.3×10^{17}	4.8×10^{17}	1.6×10^{18}	3.9×10^{16}	2.6×10^{20}
Distance (pc)	1000	71	25	260	52	2500
Calculated X-ray flux (erg cm ⁻² s ⁻¹ keV ⁻¹)	2×10^{-12}	10^{-12}	4×10^{-11}	3×10^{-11}	10^{-12}	10^{-11}
Observed X-ray flux (erg cm ⁻² s ⁻¹ keV ⁻¹)	?	?	3.8×10^{-11}	?	?	$\sim 4 \times 10^{-9}$

the primary B-star is calculated from the observed visual magnitude and an appropriate bolometric correction, and is about 10^{36} erg s⁻¹. The optical photon density is obtained using a distance of 3×10^{11} cm from the star, which is a little less than half the semi-major axis of the close binary system. Using a distance of 25 pc for Algol¹, the X-ray intensity at the Earth due to Compton scattering of the optical photons from radio electrons is about 4×10^{-11} erg cm⁻² s⁻¹ keV⁻¹, agreeing with the value of Schnopper *et al.*³.

The energy release, E , from the magnetic field twisting between the binary stars is given approximately for one turn as (see ref. 4)

$$E \sim B_s^2 a^6 / R^3 P \text{ erg s}^{-1}$$

where B_s is the surface polar magnetic field of the star, a is the radius, $2R$ is the interstar distance and P is the period of rotation. Using plausible values of $B_s \sim 30$ G, $P \sim 10^5$ s, $a \sim 2 \times 10^{11}$ cm, $R \sim 4 \times 10^{11}$ cm, we obtain $E \sim 10^{31}$ erg s⁻¹, which is approximately the energy in X-ray emission. The value of B_s is compatible with the magnetic field in the radio-emitting region if we assume a dipole field.

Table 1 shows the X-ray flux expected from the other radio binaries listed in ref. 1 using the same model. The X-ray flux observed from Cyg X-1 clearly shows that the X-ray-emitting process is different from the one considered here. Of the remaining radio binaries in the list, β Lyr seems the most promising candidate for observation.

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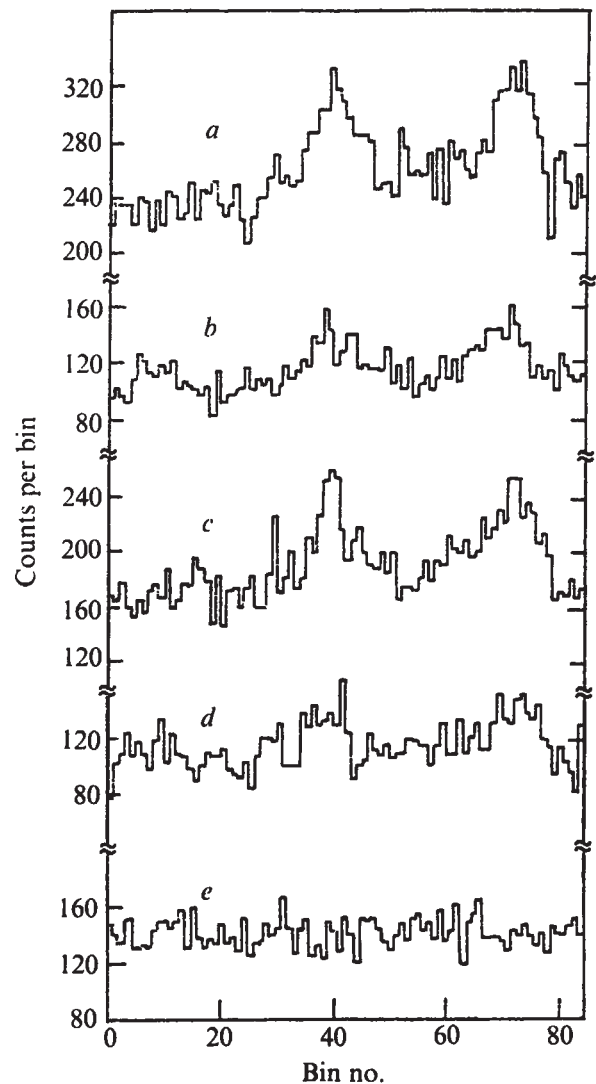
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of background data, using the period for NP0532. The pulsar period was corrected for balloon location and drift, based on optical data and calculations provided by H. Helmken of the Center for Astrophysics. The data were binned in time bins of 1/85 of the pulsar period, that is, ~ 0.3901 ms. The actual time resolution of our data, however, is 2 ms.

Fig. 1 Light curves of NP0532 on 21 June 1974 (35–116 keV). The observation intervals (in UT; seconds from June 21.0) are indicated. Scans over the Crab Nebula were made with different exposures; thus, the amplitudes of the peaks are different for the four intervals: *a*, 54130–54800 s; *b*, 71880–72200 s; *c*, 73700–74200 s; *d*, 74450–74850 s. The primary and secondary peaks occupy bins 25–45 and 66–81, respectively. A third peak (bins 5–12) is apparent ~ 12 ms before the primary in *b* and *d*. A curve for background data (*e*, 81600–82600; folded at the NP0532 period) is also given.



Transient structure in the high-energy X-ray light curve of NP 0532

ON 1974 June 21 we observed the Crab Nebula with a balloon-borne X-ray telescope (20–150 keV) with a sensitive area of ~ 700 cm². The observations took place between UT 54112 s and 78520 s. At times during this period a transitory third peak was observed in the light curve of NP0532, which we have reported earlier¹⁰. The peak comprised $2.0 \pm 0.5\%$ of the total emission from the Crab, and was detectable for ~ 5 min on two occasions (~ 40 min apart). We also report pulsed fractions in the primary and secondary peaks, and in the interpulse region.

Four light curves for the pulsar NP0532 are shown in Figs 1a–d (35–115 keV). The intervals over which data were superimposed are indicated. A fifth curve (Fig. 1e), is a superposition

Table 1 Analysis of transient light-curve peaks

Bins per group	No. of groups	$\chi^2_{\max}$		Starting bin for $\chi^2_{\max}$		Null hypothesis probability	
		<i>b</i>	<i>d</i>	<i>b</i>	<i>d</i>	<i>b</i>	<i>d</i>
6	4	16.6	13.3	5	3	0.0036	0.0169
7	4	15.5	10.3	6	2	0.0063	0.0680
8	3	19.2	9.9	5	3	0.0002	0.0392
9	3	13.6	17.0	6	4	0.0093	0.0013
10	2	12.7	9.0	5	3	0.0020	0.0196
11	2	15.1	8.8	5	3	0.0010	0.0215
12	2	14.1	11.5	5	3	0.0020	0.0066
13	2	8.3	11.0	5	2	0.0306	0.0074
14	2	6.4	8.5	5	14	0.0775	0.0295

Bins 1–24 and 82–85 in the light curves of Figs. 1*b* and *d* were used. The first column gives the number of time bins combined into larger bins (called 'groups'). The second column gives the number of groups that could be composed from the data. The third and fourth columns give the maximum value of χ^2 obtained by forming groups from adjacent time bins from Figs 1*b* and *d*, respectively. This maximum occurred when the first of these groups began with the bin number given in the fifth column (Fig. 1*b*) or sixth column (Fig. 1*d*). The sixth and seventh columns give the probability that the given values for $\chi^2_{\max}$ for the data of Figs 1*b* and *d*, respectively, would occur as statistical fluctuations in random data. These probabilities were determined by repeating the analysis procedure on 10,000 runs of computer-generated random data.

Figures 1*b* and *d* show the presence of a third pulse (bins 5–13) which leads the primary pulse by ~ 12 ms. To establish objectively a level of statistical significance for this peak, we used a Monte Carlo technique, that is, we tested for a feature of arbitrary location and width within the light curve. In applying this technique, we first calculated χ^2 for various combinations of adjacent data bins (which we called 'groups') from Figs 1*b* and *d*; a total of 28 bins (bins 1–24 and 82–85) were used in constructing the groups, covering the generally accepted 'unpulsed' portion of the light curve. Groups consisting of 6–14 bins were considered; the lowest-numbered bins were used and leftover bins disregarded when 28 was not divisible by the number of bins per group (for example, when considering groups of six bins, only bins 1–24 were used). χ^2 was evaluated for these groups, fitting the data to a constant count rate. The maximum value of χ^2 obtained in this manner is given in Table 1 for each group size used.

The same procedure was then followed on 10,000 runs of simulated data, generated assuming a constant counting rate

divided the 320 s of data used in Fig. 1*b* into two 160-s sub-intervals and established that each subinterval contributed approximately equal amounts to the feature. A duration somewhat greater than the ~ 5 min we report is also possible, as we did not observe the Crab during the ~ 4 h between the times when data for Figs 1*a* and *b* were taken. The feature was not evident ~ 20 min later, however, in a scan beginning at UT 73280 s.

We also established the pulsed fraction of the total Crab spectrum from our data, as a function of energy, by combining ~ 2 h of data taken between UT 54150 s and 78380 s on 21 June 1974 (assuming a photon number spectrum of $22E^{-2.29}$ photons $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$ for the total Crab flux⁷). Over such a long interval, the transient third peak washes out. Consequently, for this ~ 2 -h interval, we report pulsed fractions only for the primary peak, the interpulse region, and the secondary peak (Table 2). The unpulsed portion of the light curve is comprised of the sum of 28 bins: 1–24 plus 82–85.

For the period of time during which the third peak was most

Table 2 Pulsed fractions in X-ray emission from Crab (nebula and pulsar) (21 June 1974, 2 h data taken between UT 54150 s and 78380 s)

Bins	Designation	% total flux over given energy range					
		20–28	28–35	35–47	47–64	64–80	80–115
25–45	Primary peak	4.5 ± 0.8	5.6 ± 0.9	5.5 ± 0.6	5.6 ± 0.5	6.8 ± 0.6	5.9 ± 0.8
46–65	Interpulse region	3.4 ± 0.7	2.7 ± 0.8	3.4 ± 0.6	4.1 ± 0.4	3.9 ± 0.6	4.9 ± 0.8
66–81	Secondary peak	5.9 ± 0.7	7.0 ± 0.8	6.0 ± 0.5	6.6 ± 0.4	6.9 ± 0.6	9.0 ± 0.7
Σ	Total pulsed	13.8 ± 1.7	15.2 ± 1.9	14.8 ± 1.4	16.3 ± 1.0	17.6 ± 1.4	19.9 ± 1.8
							115–146
							6.7 ± 1.7
							4.6 ± 1.6
							7.2 ± 1.4
							18.6 ± 3.7

equal to the mean observed rate and fluctuations dictated by Poisson statistics. The number of runs (divided by 10,000) which produced values of the maximum χ^2 in excess of that obtained with the actual data is noted in Table 1 for each group size. These numbers are, therefore, the probabilities for a constant source producing the values of $\chi^2_{\max}$. For a group size of eight bins, only two out of the 10,000 runs of simulated data produced as large a value of χ^2 as did the data from Fig. 1*b*. Consequently, we conclude that it would be very unlikely for the Crab pulsar, if truly constant over these bins, to produce the observed data. Note that this probability level does not directly give the statistical significance of any peak present; a 'valley', for example, could as easily produce high values of χ^2 in the simulated data. The significance of the feature in Fig. 1*d* is considerably less, as 13 runs (out of 10^4) yielded larger values of χ^2 (group size of nine bins).

The width of the apparent peak (eight or nine bins) is consistent with a much narrower feature, broadened by our time resolution; of course, it is not necessarily any narrower than eight or nine bins. We were able to rule out a single, brief burst (duration $\sim$ a few ms) as the cause of the feature. We sub-

evident (Fig. 1*b*), we calculated its contribution to the flux from the Crab by assigning only the sum of bins 1–4 plus 13–24 plus 82–85 to the 'unpulsed' portion of the light curve. We find that it comprised a fraction

$$f_i = 0.020 \pm 0.005$$

of the total Crab flux (35–115 keV).

The possibility of time variability in the NP0532 X-ray light curve was first suggested by Boldt *et al.*¹, based on a factor of 1.5–2 difference between their sounding rocket measurements of the pulsed fraction in 1968 (2–10 keV) and those of Bradt *et al.*² in 1969 (1.5–10 keV). Additional peaks in the NP0532 light curve at approximately the same location as the third peak in Figs 1*b* and *d* have been reported^{3,12}. Large, infrequent variations on time scales of a few tenths of a second have been reported⁴ in the 2–6 keV X-ray flux from the immediate vicinity of the Crab, based on *Uhuru* data. Helmken⁶ has pointed out an apparent discontinuity in the light curve, obtained by subtracting the NP0532 optical light curve (normalised to the X-ray primary peak) from a composite X-ray light curve, at a point

~10 ms preceding the primary peak. He suggests a possible association with the third peak (Figs 1b and d) reported here.

For an assumed isotropic radiation pattern, the energy radiated in the third peak would be $\sim 4 \times 10^{37}$ ergs (at an assumed source distance of ~ 2 kpc). This is likely to be an overestimate, since the Earth probably lies near the plane of the pulsar rotational equator⁹. It is interesting to compare this number to the energy associated with pulsar spinups (and subsequent spindowns). For the September 1969 spinup, the fractional change in the pulsar rate has been given as $|\Delta\omega/\omega| \sim 3-9 \times 10^{-9}$ (refs 5, 8). Scargle and Pacini¹¹ argued that the accompanying total energy release from the pulsar magnetosphere was $\sim 10^{13}$ erg. The associated rotational energy gain of the rotator would have been $\sim 10^{41}$ ergs. If a similar scaling relationship were to apply for the transient third peak reported here, the resulting value for $|\Delta\omega/\omega|$ would be $\sim 10^{-14}$. Even if the pulsar spinup energy were comparable with the energy radiated as X rays, $|\Delta\omega/\omega|$ would still only be $\sim 10^{-12}$, far below the level of detectability.

In summary, the third peak is transient in nature; its duty cycle seems to be low ($\lesssim 10\%$). Further observations of this phenomenon will require extended measurements with detectors of even greater sensitivity than the ones we used.

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Diffusion time scales and accretion in the Sun

SURFACE abundances in the Sun could have been essentially caused by accretion, either of comets or of grains¹⁻³. It has been hypothesised that if surface convection zones could be smaller than is usually indicated by model calculations, then accretion could be especially important. Dust accretion by the Sun has also been suggested as the cause of ice ages on the earth⁴. Either the zone immediately below the surface convection zone is stable enough for diffusion to be important, however, or other transport processes (turbulence, meridional circulation) more efficient than diffusion, will tend to homogenise the star. Diffusion is the slowest of the transport processes and will become important when the other transport processes become

inoperative. Using diffusion theory, we can determine the minimum mass of the convection zone, if transport processes at the bottom of the convection zone are not to influence the abundances in the convection zone. If diffusion time scales are shorter than the life of the star, diffusion will modify the abundances in the convection zone. The mass in the convection zone for which diffusion time scales are equal to the life of the star on the main sequence then determines the minimum mass in the convection zone to justify neglect of transport processes at the bottom of the convection zone. I determine here that, for the Sun, this mass is between 3×10^{-3} and $10^{-2} M_{\odot}$, and give a general expression for the diffusion time scale as a function of the mass of the convection zone.

Within the convection zone, the turbulent velocities are large enough to ensure homogeneous abundances⁵. The surface abundances are assumed to be the same as those in the convection zone. If the convection zone is considered to be mixed and emptied through its bottom then letting Y be the mass fraction, in the convection zone, of the element of interest

$$\frac{d}{dt}(Y \Delta M_c) = Y w_1 \rho. \quad (1)$$

ΔM_c is the mass of the convection zone in g cm^{-2} , w_1 the diffusion velocity and ρ the density at the bottom of the convection zone (w_1 negative towards the solar interior). Equation (1) integrates to give

$$Y = Y_0 \exp(-t/\theta) \quad (2)$$

with

$$\theta = -\Delta M_c / \rho w_1 \quad (3)$$

Values of θ have been obtained⁶ in seven models of main sequence stars of mass 1–2.6 $M_{\odot}$. In the Sun, most heavy elements will diffuse downwards, unless the mass in the convection zone is $< 10^{-6} M_{\odot}$. Gravitational settling dominates in solar type stars. Aller and Chapman⁷ made similar calculations for the Sun, but neglected radiation pressure and used an incorrect thermal diffusion term (see ref. 8 for discussion). For helium in the Sun, one obtains, from equations (37), (8), and (10) of ref. 6

$$\theta = 3.6 \times 10^5 \Delta M_c^{0.545} \text{ (yr)} \quad (4)$$

or

$$\theta = 2.3 \times 10^{11} \Delta M^{0.545} \text{ (yr)} \quad (4a)$$

In equation (4), ΔM_c is in g cm^{-2} and in equation (4a), ΔM is the total mass in the convection zone in solar mass units. For helium in the Sun, equation (4) is exact to within a few per cent. For $\Delta M = 3 \times 10^{-3} M_{\odot}$, $\theta \simeq 10^{10}$ yr, and $\theta \simeq 1.7 \times 10^{10}$ for $\Delta M = 10^{-2} M_{\odot}$. The diffusion time scales for manganese are, in the Sun, nearly equal to those of He (see Table IV of ref. 6), but those of Hg are approximately 40% smaller. For transport processes to have negligible effects on surface abundances in solar type stars, the mass in the convection zone must be at least 3×10^{-3} to $10^{-2} M_{\odot}$. In solar models with the mixing length equal to the pressure scale height, the mass in the convection zone is $2 \times 10^{-3} M_{\odot}$.

Use of equation (4) to estimate diffusion time scales for helium in main-sequence stars lighter than $2 M_{\odot}$ is accurate to within a factor of 1.5, but leads to a factor of three overestimate in θ below the hydrogen convection zone of a $2.6 M_{\odot}$ star.

Diffusion time scales in the Sun are very uncertain. The uncertainty arises from our poor knowledge of ΔM_c , that is from the hydrodynamics. Equation (4) is much better known than is ΔM_c . If we use this uncertainty on ΔM_c to increase the relative importance of the accreted mass, then if $\Delta M \leq 3 \times 10^{-3} M_{\odot}$, transport processes at the bottom of the convection zone must be taken into account, and the convection zone will lose the accreted matter, through its bottom. The calculations of ref. 3 suggest that $\Delta M \leq 5 \times 10^{-4} M_{\odot}$ for accretion to be important. For $\Delta M = 5 \times 10^{-4} M_{\odot}$, diffusion reduces Z , in the convection zone, from 0.02 to 0.01. A more generous estimate of the accreted mass is given by ref. 2, and for an upper

limit of $5 \times 10^{-3} M_{\odot}$, diffusion would reduce Z by approximately a factor of 0.7. At the lower limit of ref. 3, ($\Delta M = 5 \times 10^{-5} M_{\odot}$), Z in the convection zone would be reduced from 0.02 to 0.002 or less. Transport processes at the bottom of the convection zone do not rule out completely the possibility that heavy elements in the convection zone, and so at the surface, were accreted by the Sun, but make it much less likely.

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Planetary tides during the Maunder Sunspot Minimum

CONTROL of the 11-yr and longer cycles of solar activity tides raised on the Sun resulting from the gravitational pulls of the planets has often been suggested. Rudolf Wolf and R. C. Carrington were among the early astronomers who pointed out this possibility in the middle nineteenth century¹. The close coincidence of the sidereal period of gravitationally important Jupiter (11.87 yr) with the mean period of the observed annual sunspot means (11.3 yr) raises the possibility of such a relationship; the range of possible configurations of the other planets allows a wide realm of other tidal periods and effects. In daily sunspot numbers, a small but consistent periodicity at the sidereal period of the planet Mercury has been found², and a possible 178.7-yr period in sunspots (about twice the Gleissberg cycle) has been linked with multi-planet tidal influences³. Wood and Wood⁴ have applied a dynamical theory which includes all planets but Mars to reinforce their belief in a more than chance relationship. We reconstruct here Sun-centred planetary conjunctions and tidal potentials for the AD 1645–1715 period of sunspot absence (the Maunder Minimum). These are found to be effectively indistinguishable from patterns of conjunctions and power spectra of tidal potential in the modern era of a well-established 11-yr sunspot cycle. This places a new and difficult constraint on any tidal theory of sunspot formation.

The relative tide-raising forces of the planets are as follows: Mercury 0.91, Venus 2.15, Earth 1.0, Mars 0.03, Jupiter 2.26 and Saturn 0.11, with the three outer planets negligibly small. Problems arise in any direct gravitational theory because of the apparently insufficient forces and tidal heights that are involved: the tide-raising force of Jupiter on the solar photosphere is, for example, about 10^{12} times smaller than the force of solar gravity there, making planetary tides a miniscule force¹, even in the case of a rare, nine-planet conjunction. Thus proponents of the tidal hypothesis usually revert to trigger mechanisms, which is difficult to criticise or, apparently, to test by observation.

Any tidal theory rests on the evidence of continued sunspot periodicity, and the substantiation of a prolonged period of solar anomaly in the historical past^{5,6} offers a possible *experimentum crucis*. The Maunder Minimum was the most drastic change in the behaviour of solar activity in the last 300 yr, in that sunspots almost disappeared for a 70-yr period (1645–1715), and the 11-yr cycle was probably absent. During that time, however, the nine planets were all in their orbits,

and planetary conjunctions and tidal potentials were indistinguishable from those of the modern era, in which the 11-yr cycle is well-established. This provides good evidence against the tidal theory.

We have pressed the case further by reconstructing the pattern of planetary tidal forces during the Maunder Minimum to investigate the possibility that the multiple-planet forces somehow fortuitously cancelled at the time, that is, that the positions of the slower moving planets in the late seventeenth and early eighteenth centuries were such that conjunctions and tidal potentials were at the time reduced in number and force.

Historical planetary positions were determined using a computer code supplied by R. S. Harrington of the U.S. Naval Observatory and the mean elements of planetary orbits determined by Seidelmann *et al.*⁷. Only Mercury, Venus, Earth and Jupiter were considered in final analysis because the tidal forces generated by combinations with other planets are at least an order of magnitude less than these. A conjunction was defined as a Sun-centred alignment of planets within 0.15 rad, to assure that Mercury would remain in such an alignment for > 1 d.

Daily positions were calculated from J.E.D. 2296445 to 2451065 (about AD 1575–2000) and all two-, three-, and four-planet conjunctions noted. The results are shown in Fig. 1, with the history of the annual mean sunspot number. Binary conjunctions are omitted in the presentation. Higher order conjunctions exhibit subtle and beautiful symmetries and beat patterns, but there is no striking dissimilarity between the time of the Maunder Minimum and any other period shown.

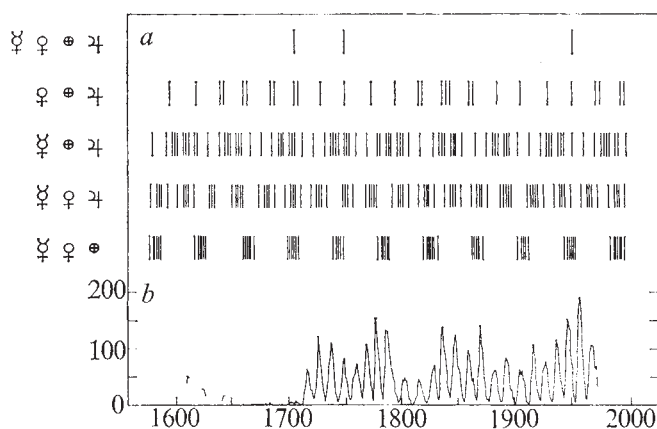


Fig. 1 *a*, Multiple planetary conjunctions (alignment within 0.15 rad) AD 1575–2000. *b*, Mean sunspot number⁸.

The failure of planetary conjunction patterns to reflect the drastic drop in sunspots during the Maunder Minimum casts doubt on the tidal theory of solar activity. Yet this test is only a qualitative judgement of a pattern and not demonstrably conclusive. A more quantitative test comes from an examination of the tidal potentials, which are proportional to

$$P(\mathbf{x}) = \sum \frac{M_i}{R_i^3} \left(\cos^2 \phi_i - \frac{1}{3} \right) \quad (1)$$

where M_i is the mass of planet i , R_i is its distance from the centre of the Sun, and ϕ_i the angle, seen from the centre of the Sun, between the planet and the point $\mathbf{x}$.

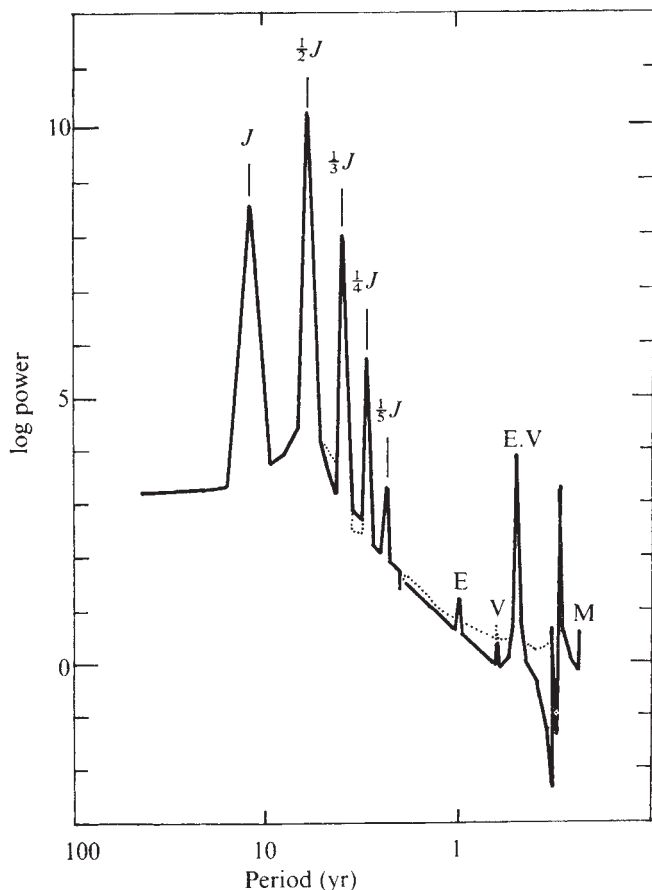


Fig. 2 Fifty-year power spectra of the tidal potential of planets on the rotating equator of the Sun (equation (1)). —, AD 1925–1975; ·····, which appears only where different from heavy line, AD 1650–1700. No differences appear in the major tidal peaks or in any other significant feature of the spectrum for these or any other epochs we examined. Major peaks correspond to fundamental and harmonic periods of the major tide-producing planets, identified by letter: J, Jupiter; E, Earth; V, Venus; M, Mercury.

We calculated a daily value of the tidal potential defined above at a point fixed on the rotating equator of the Sun, for AD 1450–2000. The period was then divided into sample intervals of about 50 yr, overlapped at 25-yr intervals, and the power spectrum of tidal potential calculated for each 50-yr sample using a fast Fourier transform. An example of the power spectrum of tidal potential is shown in Fig. 2. Principal peaks in the spectrum correspond to orbital periods of each planet and the harmonics thereof. (L. Auer has pointed out that each harmonic corresponds to the frequency of an epicycle in a Copernican decomposition of the orbit.) The usual means of dealing with the functional periodicity implied by Fourier techniques, such as tapering the end points to the mean, were inadequate in this case, since the longest periods of interest are a large fraction of the 50-yr sampling interval. This problem was avoided by adjusting the length of the sampling interval so that the potential function and its derivative were equal at the end points, hence the implied periodic function was continuous.

To compare the power spectra of tidal potential in different intervals we selected as a standard interval the modern period AD 1925–1975 (Fig. 2) and computed the ratio of the spectrum of this standard interval to all others from AD 1450 to 2000. We found that the power in each of the major peaks of tidal potential (notably the dominant first three harmonics of Jupiter) have not changed perceptibly in the last 500 yr. Also, there was no appreciable change in the power at any frequency during the years of the Maunder Minimum. In Fig. 2 we compare power

spectra for the 1925–1975 period with that of the Maunder Minimum and find them effectively indistinguishable.

A few exploratory calculations showed possibly significant changes in the spectrum of tidal potentials over much longer periods (several millennia) which seem associated with known, secular changes in the orbital elements of the planets. For example, the eccentricity of Jupiter's orbit changes by about 0.1% per century⁷. There is no evidence, however, that these secular changes are associated with possible secular changes in solar activity.

Okal and Anderson⁸ in examining the same problem computed a single power spectrum of the planetary tidal potential (or rather, its maximum value in longitude) for 10-d intervals from roughly AD 1800 to 3600. This sample, much longer than ours, defined the frequency of power peaks rather precisely. They found only one peak near that of the sunspot cycle, at the orbital period of Jupiter, 11.86 yr. They compared this with a power spectrum estimate on Wolf sunspot numbers⁹ which showed peaks at 11.0 and 9.8 yr in this region, and reasoned that the discrepancy between 11.86 and 11.0 yr sufficed to rule out tides as a driving force for the solar cycle.

Peaks in the maximum entropy spectra derived by Cohen and Lintz⁹ vary with the details of the input function: drastically in amplitude, and less in frequency. Their published examples suggest that the method of computation and data selected can shift the location of peaks near 11.0 yr period by at least ± 0.1 yr, and amplitudes by a factor 3. This is consistent with the difficulties with spectral decomposition by the maximum entropy method^{10,11}. Moreover, the period chosen by Cohen and Lintz includes all sunspot numbers from 1750 onward. Waldmeier¹² casts doubt on the quality of the early sunspot data as does Eddy⁶, who points out that controlled observation of sunspots did not commence until about 1852. Thus, since the power peaks derived by Cohen and Lintz were based in part on questionable data, the significance of an apparent discrepancy between these sunspot power peaks and those of averaged tidal periods seems less than conclusive, and in need of the support presented here.

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Order and disorder in weakly ionised plasmas

WE report the observation of novel radial structures for discharges in pure helium and argon gases in a magnetic field. The structure depends on the angle between the discharge tube axis and the magnetic field direction, a dependence which supports the idea, previously proposed, that gaseous plasma energised in specific conditions can have regions in which the electrons have an ordered spatial distribution.

The weakly ionised, high-frequency, electrodeless discharges were energised at powers between 500 W and 1 kW coupled to

the discharge from an amplifier via a 143-cm long quarter-wavelength twin transmission line; this was connected to two copper bands, 14 cm apart, placed closely around the outside of the discharge tube. The discharge was maintained continuously between the bands and in a position centrally between a pair of Helmholtz coils in a horizontal, uniform magnetic field.

Optical flats sealed to each end of the hard glass tubes made it possible to view the discharges axially. The tubes were 70 cm long, and of internal radius 1.84 cm. A pattern of luminous and non-luminous plasma regions was observed, arranged in coaxial symmetry, the complexity of the pattern depending on the magnitude of B . At $B = 0$ (Fig. 1a) shows the simplest pattern for helium gas at 0.25 torr pressure. At $B = 1,000$ G, (Fig. 1b) besides the outer dark shell, a central non-luminous plasma was observed, the outer luminous region now being enclosed between the two dark regions. At $B = 1,750$ G the inner dark region was larger in diameter, and a further plasma region, this time luminous and blue in colour was seen on the tube axis, inside and much shorter in length than the inner dark region (Fig. 1c).

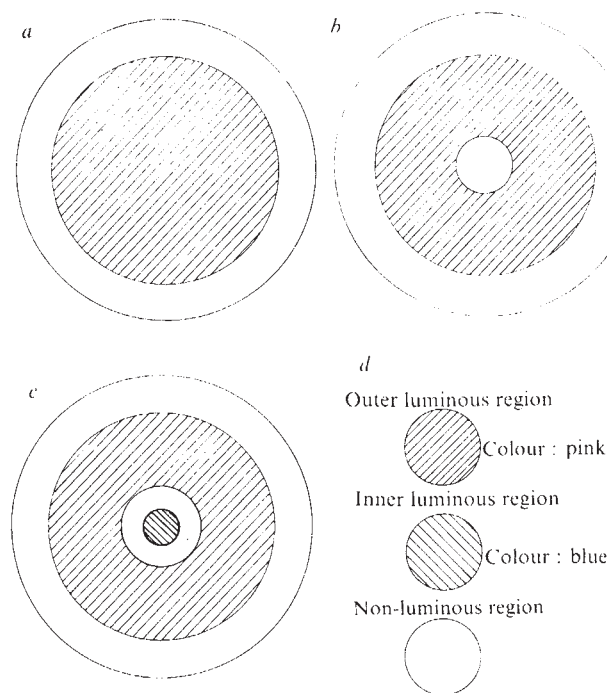


Fig. 1 Plasma regions: a, $B = 0$; b, $B = 1,000$ G; c, $B = 1,750$ G; d, spectral analysis of helium light and key to figures.

Visible lines identified	
Wavelength (nm)	Transition
438.8 (w)	$5^1D \rightarrow 2^1P$
443.8 (w)	$5^1S \rightarrow 2^1P$
447.1 (s)	$4^3D \rightarrow 2^3P$
471.3 (s)	$4^3S \rightarrow 2^3P$
492.2 (s)	$4^1D \rightarrow 2^1P$
501.6 (s)	$3^1P \rightarrow 2^1S$
504.8 (s)	$4^1S \rightarrow 2^1P$
587.6 (vs)	$3^3D \rightarrow 2^3P$
667.8 (w)	$3^1D \rightarrow 2^1P$
706.5 (vw)	$3^3S \rightarrow 2^3P$
w = weak	
vw = very weak	
s = strong	
vs = very strong	

Figure 1d gives an analysis of the spectral emission from the outer visible region. The calculated electron temperature for this region is 85,000 K, and the measured average electron density for all regions was $7 \times 10^{10} \text{ cm}^{-3}$.

Experiments in which the helium discharge tube was suspended from a spectrometer base and rotated in the hori-

zontal plane showed that at given field strength, the diameters of the central regions were critically dependent on θ , the angle between the tube longitudinal axis and the direction of B ; this is shown in Fig. 2a, in which the diameters are plotted against θ for B constant in magnitude ($B = 1,750$ G). Figure 2b shows how the diameters of both inner and outer regions vary with the field strength, keeping $\theta = 0$. Given the results of Fig. 2a, it follows that there is a critical value for θ , θ_c , for which the central regions disappear or reappear as θ is increased or decreased, respectively, at fixed B . Figure 3 is a plot of θ_c against B .

In argon gas, for the range of field strengths of the experiment, the central regions differ from those for helium. In argon at 0.37 torr pressure, for example, no luminous central region is observed, and the dark central region is just beginning to appear at $B = 1,650$ G.

The central plasma structure (Fig. 1b and c) and its dependence on θ , (Fig. 2a), are significant evidence for the presence of order in the non-luminous regions. These regions are believed to be occupied by oscillating electrons which have very little energy and are therefore not capable of exciting gas atoms, so that the regions are non-luminous. The basic theory of the interaction between low energy electrons and large numbers of gas atoms and molecules in which they are immersed, was given by Fermi¹. According to his analysis, in which the electrons are treated as electron waves, stationary states form under certain conditions. For the present purpose we assume that the oscillators are formed from pairs of electrons, or aggregates of pairs of electrons, and the requisite conditions for stationary states relate the electron number density, n , the neutral atom

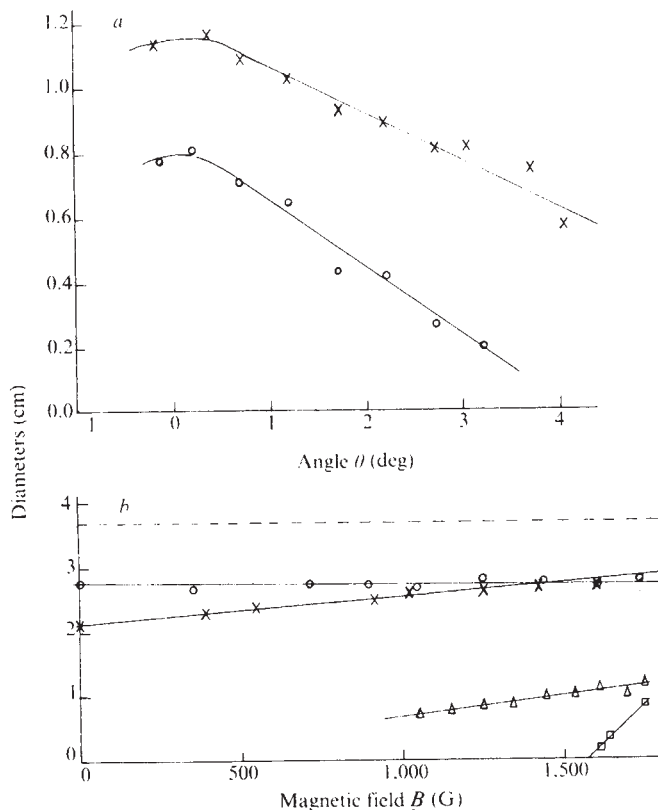


Fig. 2 a, The diameters of the central regions against θ ($B = 1,750$ G). $\times$, Non-luminous region. $\circ$, Luminous region. b, The diameter of the plasma regions against B ($\theta = 0$). — — —, Tube internal glass wall diameter; $\circ$, diameter of the outer region junction (0.25 torr helium); $\times$, diameter of the outer region junction (0.67 torr argon); $\triangle$, diameter of the inner non-luminous region (0.25 torr helium); $\square$, diameter of the inner luminous region (0.25 torr helium).

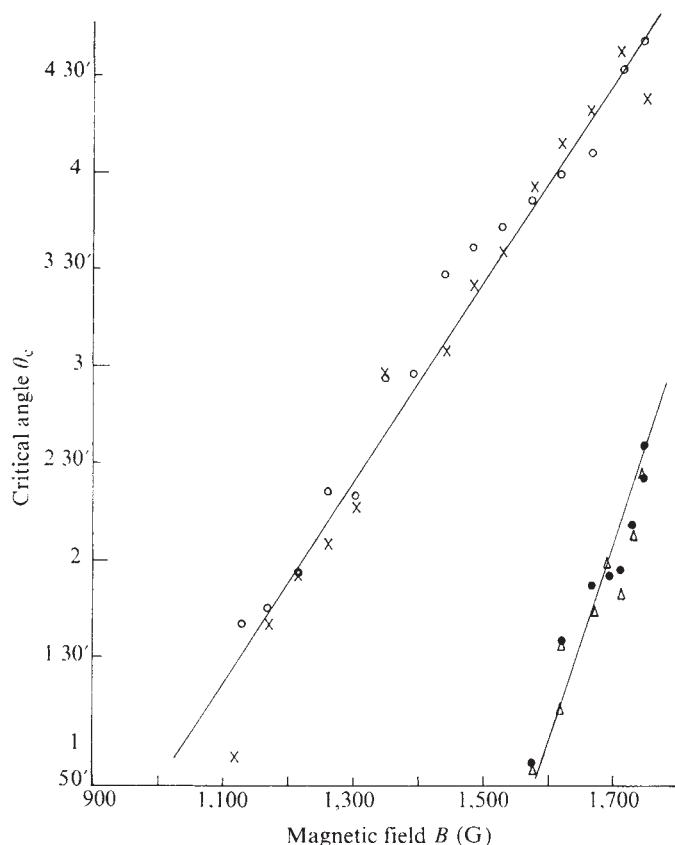


Fig. 3 The critical angle, θ_c against B (central regions). $\times$, Non-luminous region (θ increasing); $\circ$, non-luminous region (θ decreasing); $\bullet$, luminous region (θ increasing); Δ , luminous region (θ decreasing).

number density, N , and atomic constants². When $B = 0$ at low pressures the conditions occur near the tube walls, where n is smaller than on the tube axis; when B is raised to > 1000 G, however, the magnetic energy of the oscillators becomes significant and the conditions for stationary states are changed. In helium, the conditions are found not only near the tube walls but also at higher electron densities on the tube axis where the energies of oscillations are higher. A non-luminous region then appears on the tube axis. In argon, on the other hand, the potential energy of the interaction of the oscillating electrons and the gas atoms is negative rather than positive as in helium¹, a difference between the gases which results in the differences in the variation of the outer dark shell radius with B (Fig. 2b), and in the central structure. These are the lines on which an interpretation of the phenomena is believed to be possible.

Both the relatively sharp boundaries between the luminous and non-luminous regions, and the dependence of the central structure on θ are considered to show that the oscillators form a close-packed matrix, capable of reflecting fast-moving free electrons from the luminous regions, and of having anisotropic properties typical usually of crystalline substances.

The idea that ordered plasmas are sometimes formed in weakly ionised gases was prompted, not by the present visual studies, but from a prolonged study³ of the properties of rare gas afterglow plasmas. From these studies we inferred a division of the afterglow plasma into two regions for $B = 0$; at the start of electron density decay, we deduced that the junction of the two regions should be near the point on the tube radius where n has fallen to one half of its value on the axis. Assuming a simple Bessel function variation of n with radial position, this prediction from the afterglow work is that the junction between the two outer regions should occur near a diameter of 2.29 cm (in good agreement with Fig. 2b for $\theta = 0$).

Other measurements can be interpreted successfully using the model; for example, the measurement of forces of weak elastic constraint acting on plasma electrons⁴, the very fast plasma decay rates observed at $B = 0$, the anisotropic properties of afterglows in a magnetic field⁵, and the resonances attributable to electron spin-flip in a magnetic field, can all be interpreted in terms of the ordered electron model. The anisotropic properties of afterglow plasmas are good evidence of correlation between the afterglow phenomena and the currently studied continuously excited plasmas. An examination of the value for θ required for the transition from slow to fast afterglow decay rates in helium at 0.25 torr pressure as a function of B reveals that when θ is just large enough for the fast decay rate to occur, the central dark plasma region in the excited plasma preceding the afterglow is just disappearing (Figs 2a and 3).

It is interesting to consider an extension of the idea of spatially ordered electron oscillators to some naturally occurring plasmas. Such an extension needs a change of emphasis in the theory, away from electrons as particles translating with random motion in the space between the other plasma particles, to electrons as waves occupying and propagating through the space containing the ionic, atomic and molecular fields.

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Metal accumulation rates in sediments from Mid-Indian Ocean Ridge and Marie Celeste Fracture Zone

THE existence of metalliferous sediments on mid-ocean ridges is well documented¹ and the general conclusion is that they form by precipitation from submarine hydrothermal solutions. Pacific and Atlantic Ocean sediments have been studied in detail²⁻⁶. We describe here material from the Indian Ocean Ridge, collected during cruise 8/75 of RRS Shackleton, and compare metal accumulation rate data for ridge-crest and -flank sediments with those from other regions. The metalliferous sediments are present on the Mid-Indian Ocean Ridge, particularly near the Triple Junction^{7,8}, and Bostrom and Fisher⁷ suggest that "there is a complete belt of iron-rich sediment along the entire length of the Indian Ocean-Pacific Ocean active-ridge system". We have found ferromanganese encrustations on basalts from the median-valley wall and have recognised what are probably sulphides in decorated vesicles of basalts. The metal accumulation rate data argue against a hydrothermal source of metals in the ridge sediments, however, and suggest that metalliferous sediment accumulations in the Indian Ocean may be more local phenomena than previously realised.

Sample locations and bathymetry of the study area are shown in Fig. 1. The Marie Celeste Fracture Zone is sharply defined by topography, seismicity and magnetics⁹ and is one of the larger fracture zones offsetting the Mid-Indian Ocean Ridge. The half-spreading rate of the ridge is ~ 1.8 cm yr⁻¹ and the

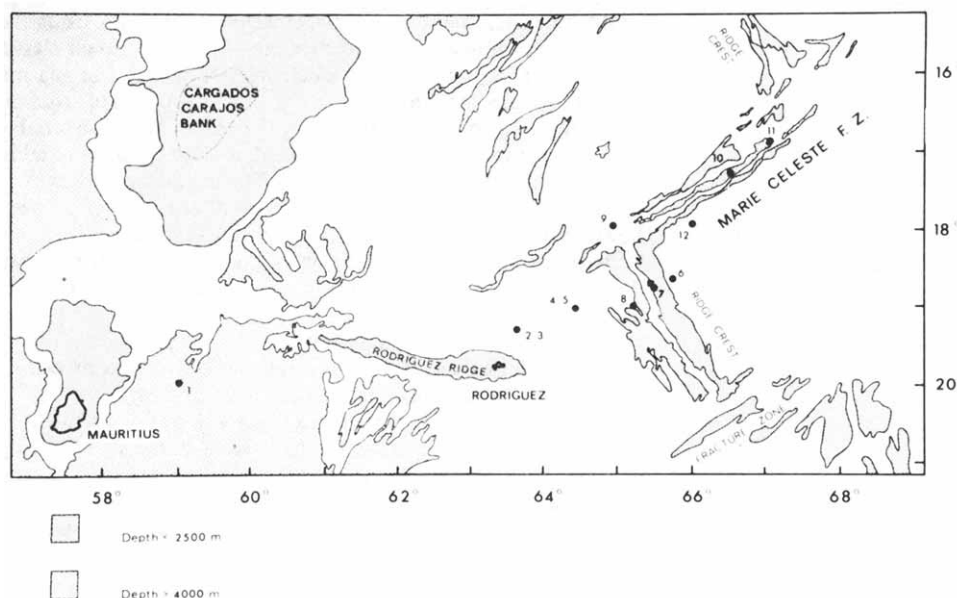


Fig. 1 Core location map showing bathymetry in region of Marie Celeste Fracture Zone. ●, Cores; ■, manganese crust.

ridge offset is 220 km (ref. 9). The maximum depth of the fracture zone is reported as 5836 m (ref. 10). The region shown in Fig. 1 seems to be a likely location for hydrothermal solutions and associated metal-rich sediments⁵.

The sediment type ranges from siliceous lutite of low to moderate carbonate content, to calcareous lutite (~90%

of a section of core 12 at 18–20 cm which gave an age of approximately 20,000 yr. The rate of $\sim 1 \text{ cm } 10^{-3} \text{ yr}^{-1}$ was confirmed by oxygen isotope stratigraphy (N. Shackleton, personal communication, 1976) and is in agreement with other estimates and measurements for this region^{10,15,16} and with world averages for carbonate sedimentation¹⁷. Metal accumula-

Table 1 Typical analyses of ridge-crest and flank sediments and sediment from the Marie Celeste Fracture Zone

%	GC2 (0–2 cm)	GC4 (0–2 cm)	GC7 (8–9 cm)	GC10 (0–5 cm)*	GC12 (0–2 cm)
SiO ₂	1.75	1.58	1.61	18.0	3.18
TiO ₂	0.024	0.029	0.022	0.32	0.040
Al ₂ O ₃	0.41	0.40	0.47	4.6	0.60
Fe ₂ O ₃	0.58	0.72	0.62	5.92	1.31
MnO ₂	0.093	0.119	0.094	0.71	0.178
MgO	0.41	0.37	0.68	2.71	0.52
CaO	50.9	50.6	49.7	31.3	48.2
K ₂ O	0.080	0.089	0.093	0.53	0.113
P ₂ O ₅	0.10	0.22	0.13	—	0.22
Na ₂ O	1.36	0.90	1.71	3.62	1.40
L.O.I.	42.93	42.59	42.48	30.78	41.27
Total	98.61	97.57	97.62	98.49	97.06

*From the fracture-zone floor. SO₄, Cl not determined.

CaCO₃) in relation to the depth of deposition. Physical parameters (texture, colour, etc.) are very similar between and within the carbonate cores, suggesting that the three cores studies in detail are representative of the remainder. One core collected from the fracture-zone floor consists of siliceous lutite overlain by 110 cm of red clay. Chemical and microscopic comparisons with the carbonate sediments suggests that the fracture-zone core represents a condensed sequence deposited below the carbonate lysocline.

There is no significant change in major-element chemistry with depth, except as a consequence of a 3–5-cm glass horizon which occurs between 37 and 62 cm in individual cores, and the compositions listed in Table 1 are typical of these sediments. The volcanic glass is acidic (refractive index ~ 1.50) and fine grained, and probably derives from Indonesia (G. Walker, personal communication, 1976). Also present are basalt fragments containing decorated vesicles of a type already reported^{11–13}. Figure 2 shows a scanning-electron micrograph of a vesicle revealed by the fracture of a basalt fragment during sample preparation. Such decorations have been interpreted as sulphides^{11–13} and their occurrence in the sediment is supported by evidence of sulphide mineralisation in Indian Ocean ridge basalts¹⁴.

Mean sedimentation rate was obtained by radiocarbon dating

tion rates (Table 2) were calculated by age correlation using the acidic glass horizon. Comparison with accumulation rates in normal pelagic sediments and in hydrothermal sediments from the East Pacific Rise shows that metals are accumulating at these locations on the Mid-Indian Ridge at rates commen-

Fig. 2 Decorated vesicles in a basalt fragment from a carbonate core. The wall decorations are probably sulphides.

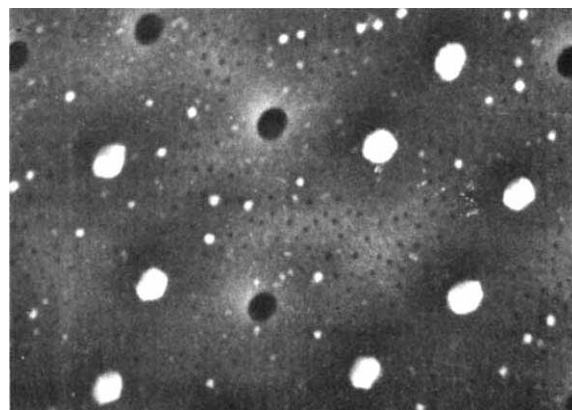


Table 2 Element accumulation rates ($\text{mg cm}^{-2} 10^{-3} \text{ yr}^{-1}$) in Indian Ocean, normal pelagic, and East Pacific Rise sediments

	Indian Ocean Marie Celeste Fracture Zone	Ref. 23	Pelagic sediments Ref. 18	Ref. 24	East Pacific Rise sediments Ref. 1	Ref. 3
Ti	0.08–0.2	0.2–0.9	—	0.4	0.074	—
Al	1.5–2.6	4–18	—	6	0.72	—
Si	4.2–9.6	—	—	17	—	55
Fe	2.4–5.2	3–9	—	4	63	110
Mn	0.4–0.7	0.3–1.0	0.2–3.2	4	24	35

surate with the lower rates of pelagic sedimentation. Hence, no unusual processes are responsible for metal deposition in these sediments. The deposition rate for manganese ($0.4\text{--}0.7 \text{ mg cm}^{-2} 10^{-3} \text{ yr}^{-1}$) is similar to that in nodules ($0.2\text{--}1.0 \text{ mg cm}^{-2} 10^{-3} \text{ yr}^{-1}$) (ref. 18). A slow, uniform precipitation of Mn over the sea floor has been inferred from the similar rates of Mn accumulation in nodules and sediments from all oceans¹⁸ and suggests that the metals accumulating on the Mid-Indian Ridge are authigenic in origin. The ratio $\text{Al}/(\text{Al} + \text{Fe} + \text{Mn})$ for these sediments (0.24–0.35) is, however, within the range used to classify Mid-Indian Ridge sediments as metal rich and of volcanogenic origin^{1,7,8}. The sediments we examined are not of volcanogenic origin although they conform to this classification. It is clear that metal–aluminium ratios do not necessarily indicate regions of metalliferous sediment deposition and must be used with caution in this respect.

Despite the extensive nature of hydrothermal deposits on the East Pacific Rise many metal-rich deposits are of a very localised nature^{2,5,6,19–21}. Our results do not disprove the existence of metal-rich exhalations and deposits within the area studied, but suggest that such deposits on the Mid-Indian Ridge are probably local and certainly less widespread than sometimes supposed. We made many attempts to sample sediment from within and on the flanks of the median valley in the hope of detecting hydrothermal material. No sediment was recovered, however, but, instead, the corer sampled a fragmented $\sim 15 \text{ mm}$ thick ferromanganese encrustation on basalt.

The encrustation contains 20.0% Fe, 12.5% Mn, 0.11% Cu, 0.081% Co, 0.14% Ni and 0.060% Zn. For manganese to be accumulating at a rate $\gg 1 \text{ mg cm}^{-2} 10^{-3} \text{ yr}^{-1}$ the oceanic crust at this point must be $\ll 120,000 \text{ yr}$ old. As the encrustation was recovered from the median valley wall this is possible and it is just conceivable that this material may contain a component of local hydrothermal origin. Its overall composition, however, is unlike that of hydrothermal manganese crusts from the Mid-Atlantic Ridge median valley⁶, The Galapagos spreading axis² or the Romanche Trench⁵, and is very similar to S.W. Indian Ocean nodules²². In the absence of other evidence, therefore, it must be regarded as a hydrogeneous deposit.

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Estimation of gas-releasing efficiency of erupting magma from ^{226}Ra – ^{222}Rn disequilibrium

VOLATILE components of volcanic magma have an important role in its explosive eruption¹. The volatiles may be partially released from the erupting magma into the air, during eruptive events, and the ratio of the released to the original amount may be a possible determinant of the mode of explosive eruption. Direct measurements of these quantities, however, are extremely difficult; fresh ejecta are rarely obtained. The Sakurajima Volcano, Kyushu, Japan, erupted explosively on 30 October 1975, and discharged pumices and ash-like materials, some of which were later accessible. We report here a change in the measured radioactivity of pumice, possibly due to radon release.

Both types of ejecta were collected immediately after the eruption by the staff of the Sakurajima Volcano Observatory, Kyoto University. Within 48 h these samples were subjected to non-destructive γ -ray spectrometry, using a Ge(Li) semiconductor detector, at the Radioisotope Centre, University of Tokyo, to determine their contents of natural short-lived radionuclides (Table 1).

Gamma-ray spectrometric data so far obtained for some of the products from major eruptive episodes of the Sakurajima show no significant change in the U- to Th-series ratio of magma for the last few hundreds of years (Sato and Sato, in preparation). These data are included in Table 1 for comparison. Compared with the 'ash' sample and historical lavas, the 1975 pumice sample showed a marked deficiency in ^{214}Bi . A measurement performed on the same sample 1 month later showed that the specific activity of ^{214}Bi increased to a value similar to those for other materials listed in Table 1; the ascendant short-lived nuclides in the U-series which had been depleted through the eruptive event could have increased during the period. This observation agrees with the differing origins of the two types of ejecta: the pumice represents fragments of the erupting magma whereas the ash-like material could be composed of fragments of rocks pre-existing around the crater.

The significant degree of vesiculation of the pumices shows that a substantial amount of gases was discharged. The gaseous radioelement, radon, may have been expelled at this stage of eruption. One of radon's isotopes, ^{222}Rn (half life: 3.8 d), requires about 1 month to increase to an

Table 1 Th- and U-series radioactivity in some products of Sakurajima Volcano (10^{-13} Ci g $^{-1}$)

Sample	Th-series (from ^{228}Ac and ^{208}Tl)	U-series (from ^{214}Bi)	U-series Th-series
Pumice, 30 October 1975; andesite*	6.7 ± 0.8	1.84 ± 0.61	0.28 ± 0.09
'Ash', 30 October 1975; andesite*	5.7 ± 0.2	3.8 ± 0.2	0.67 ± 0.04
Lava, 1914; andesite	5.7 ± 0.2	4.4 ± 0.3	0.77 ± 0.06
Lava, 1776; andesite	8.5 ± 0.7	5.6 ± 0.5	0.66 ± 0.08
Pumice, Moeshima; dacite	5.7 ± 0.2	3.8 ± 0.2	0.67 ± 0.04

*Measurements were made within a few days of their fall.

amount in equilibrium with the parent, ^{226}Ra , and is expected to show its transient deficiency for several days after the event. This could explain the lowering of the U- to Th-series ratio observed for the pumice (Table 1). The ratio is, therefore, regarded as being indicative of the ^{226}Ra – ^{222}Rn disequilibrium.

In subsequent eruptive activities (a detailed account will be published later), the volcano ejected, on 13 May 1976, a considerable amount of pumice. Figure 1 shows U- to Th-series radioactivity ratios for this material, and shows that the U- to Th-series ratio of the 1976 pumice was again significantly lowered for several days after the eruption, then increased to a value close to those of earlier lavas (Table 1).

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Effects of inbreeding on cognitive performance

THE few studies^{1,2} in which the effects of inbreeding on cognitive performance have been examined revealed that offspring of first-cousin marriages had lower IQ scores than offspring of unrelated parents. These studies were, however, performed in societies where the population engaging in such marriages is a small (1%, 6%)^{2,3} and unrepresentative proportion of the total population. Possible confounding of the effects of inbreeding with the effects of other intelligence-related variables such as socioeconomic status may lead the effects of inbreeding to be overestimated². Unfortunately statistical control may either over- or under-correct for the correlates of the independent variables, leaving one in doubt about the true effect of inbreeding. I have now examined the effects of inbreeding on cognitive performance in an Arab population with a high rate of consanguineous marriage which minimised the distortions due to non-genetic variables. I show here that offspring of unrelated parents performed better than offspring of first-cousin marriages in intelligence and achievement tests administered at grades 4 and 6. The lowest level of performance and a higher variance were found for offspring of double-cousin marriages. The inbreeding depression found in this study is consistent and cannot be explained by the effects of socioeconomic status. I drew a nationally representative sample of 3,203 children in grades four and six (approximate ages 10 and 12 yr) of the Arab educational system in Israel. This sample constitutes about 10% of the total population in these grades and includes only normal (not retarded) children. Column 1 in Table 1 shows the division of the subjects according to grade level and consanguinity of the parents. A first-cousin marriage is between children of siblings. Children of first cousins have, on average, 1/16 pairs of genes by common descent. Double first cousins are children of siblings married to unrelated siblings. When they marry, their children have, on the average, 2/16 pairs of genes by common descent.

A study of Arab education in Israel revealed that the frequency of consanguineous marriages in the Arab population is about 34%. The Arab community encourages such marriages and therefore the group that intermarries should be a representative sample of the population. Double first-cousin marriages are hardly known in Western Society but

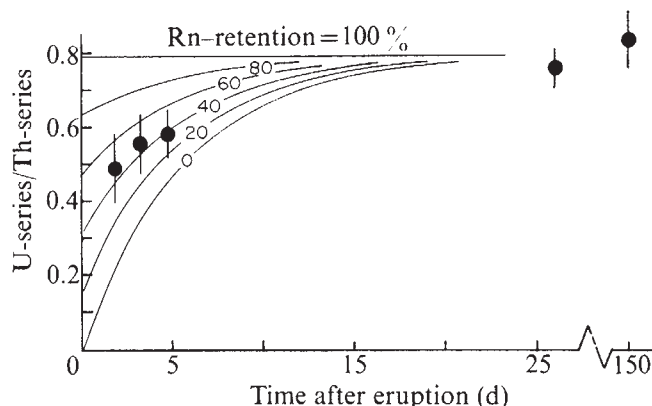


Fig. 1 Growth of U- to Th-series radioactivity ratio observed for the 1976 pumice from Sakurajima. Growth curves are based on the assumption that a ^{226}Ra – ^{222}Rn equilibrium was established in the magma before the eruption. The values are plotted against the mid-points of counting times.

The disequilibrium observed above may also be due to radon release at the moment of eruption. If a ^{226}Ra – ^{222}Rn equilibrium is assumed to have been established in the magma before the eruption, the growth of ^{222}Rn in the pumice should indicate its re-establishment. The data are expected to fit a growth curve derived from two parameters: (1) the observed U- to Th-series activity ratio of pumice at the equilibrium and (2) the percentage of unreleased radon in the erupting magma. In Fig. 1 are shown the growth curves for postulated values of (2), and, on this basis, the data correspond to a 50–60% release of radon.

The behaviour of radon in an erupting magma may be different from that of major volatiles. It would be possible, however, to characterise explosive eruptions by extending the method presented here to other fresh pyroclastic materials.

We thank K. Kamo, Disaster Prevention Research Institute, Kyoto University for the samples, and M. Takeda,

exist to a significant degree (about 4%) in my sample population. Their popularity is no doubt partly explained by economic considerations concerning the custom of 'bride price'. When a brother and sister of one family marry a brother and sister of another, there is no need for the groom to pay the bridal price.

It is reasonable to assume that other consanguineous marriages—between, for example, second and third cousins—exist in our sample. However, only three alternatives were given to the question on blood relationships (unrelated, cousins and double cousins) and, consequently other consanguineous marriages were probably classified by the respondents as one of the two latter categories, especially first cousin marriages. This would tend to make the real inbreeding coefficients somewhat lower than the above coefficients (1/8, 1/16). We can also assume that, since prolonged inbreeding increases the coefficient of inbreeding³, the inbreeding coefficient of the control group is not zero. But prolonged inbreeding has also occurred for the inbred groups, so it is safe to assume that there exists an increasing order of the degree of inbreeding, from the control group (of outbred children) to the first cousins' offspring and to the double cousins' offspring.

Table 1 Consanguinity and socioeconomic status of subjects

	Number of subjects	Father's education (mean yr)	Mean SES†
Grade 4			
First cousins	503	5.6	9.7
Double first cousins	71	5.1	9.1
Others (unrelated)*	1,054	5.2	9.4
Total sample	1,628	5.3	9.5
Grade 6			
First cousins	467	5.4	9.5
Double first cousins	54	5.0	9.1
Others (unrelated)*	1,054	5.0	9.4
Total sample	1,575	5.1	9.4

*In a population with a high rate of consanguineous marriages the so-called 'unrelated' group probably does not have a zero inbreeding coefficient (see text).

†Socioeconomic status.

Socio-economic status (SES) of the groups was defined as an index of the following four components: (1) father's plus mother's level of education; (2) father's profession; (3) room density; and (4) physical home conditions. Each of the four components was divided into four graded categories. SES was defined as the sum of these four components, thus ranging from four to sixteen, with each of the four components being of equal weight. Table 1 shows that unrelated parents have somewhat lower SES than the first cousins. The double first cousins have lower SES than the unrelated group but the difference is less than 0.1 s.d. As the very small differences in SES favour the first cousins as compared with the other two groups, any reduction in performance found in the inbred groups cannot be explained by differences in SES.

Three mental ability tests and four achievement tests were used. As the genetic component is assumed to be larger in informal learning (ability tests) than in specific knowledge formally taught in the classroom (achievement tests) I expected inbreeding to have a smaller effect on performance in achievement tests than in ability tests. The mental ability tests included (1)a, a verbal general knowledge and comprehension test and b, a verbal test to find the 'odd one out'. Both tests were equally weighted to construct a single verbal IQ score. (Alpha reliability coefficient is 0.93, correlations averaging 0.70 with achievement tests and teacher's evaluation.) (2) A shortened version of Raven's Progressive Matrices⁶ was used to measure non-verbal ability. (3) A substitution test that, unlike the other two, was given under a strict time limit and was used to measure associative learning. The four achievement tests

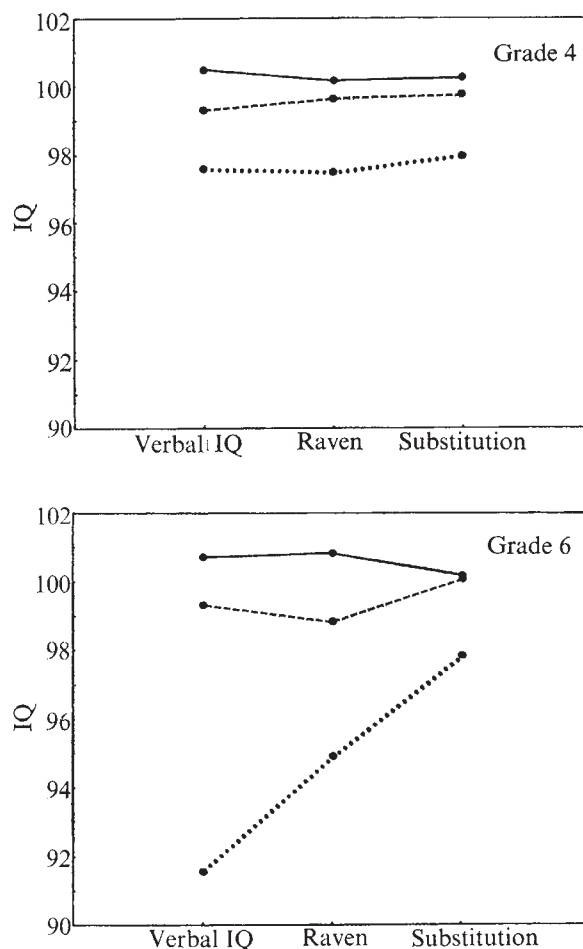


Fig. 1 Mean IQ scores on the three mental ability tests to parental consanguinity, for grades 4 and 6. — Not related, --- cousins, double cousins.

were constructed in accordance with the specific school curricula of each grade (Arabic, Hebrew, mathematics and science). The alpha reliability coefficients range from 0.80 to 0.91 and the correlations between the four tests average about 0.70. All tests were administered to groups of children and given simultaneously in all the sample classes.

Table 2 presents the mean scores and standard deviations for the mental ability and achievement tests, according to grade and parental consanguinity. Figure 1 shows the IQ scores on the three mental ability tests for the different consanguinity groups, separately for each grade. Figure 2 shows the *t* scores of achievement tests (with a mean of 50 and s.d. of 10 for each test).

All the results follow the expected pattern and the order of performance level of the three groups is consistent. Outbred children achieved the highest level of performance and offspring of double cousin marriages achieved the lowest. Thus, an inbreeding depression is suggested in both grades and in all the tests.

The results in Table 2 show a tendency towards higher variance in the double cousin group. This is the case in 13 out of the 16 possible comparisons; the result is statistically significant in five of the comparisons, all of which are in grade 6 (general knowledge, finding the odd one out, Arabic, Hebrew and science).

The inbreeding depression shown in Table 2 and Figs 1 and 2 is too modest to warrant changes in educational policy, but the consistency of the effect for the different tests in both grades strengthens the argument that its cause is indeed genetic. Surprisingly, there was no difference between performance in achievement and ability tests: inbreeding

Table 2 Mean scores and standard deviations of mental ability and achievement tests according to parental consanguinity and grade level

Grade	Parental consanguinity	Mental ability tests								Achievement tests							
		Verbal ability															
		General knowledge (51)*		Finding odd-one-out (25)*		Raven's Matrices (28)*		Substitution (144)*		Arabic (58/57)†		Hebrew (51/51)†		Mathematics (39/40)†		Science (29/30)†	
		Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
4	'Unrelated'	32.2	8.3	12.8	5.6	8.8	5.1	53.5	24.4	37.1	11.9	21.8	7.8	21.2	7.9	14.7	5.1
	First cousins	31.5	8.9	12.3	5.8	8.6	5.1	52.7	28.2	36.3	12.0	21.3	7.9	20.8	8.4	13.5	5.3
	Double first cousins	31.0	9.3	11.4	5.9	7.9	5.1	49.6	23.7	34.4	12.9	21.1	8.1	20.1	8.9	13.3	5.3
6	'Unrelated'	38.4	8.3	16.4	5.9	13.1	6.1	70.5	27.0	37.9	10.6	31.4	10.2	18.6	8.7	17.6	5.7
	First cousins	37.6	8.2	15.8	6.1	12.3	6.2	70.4	27.0	37.4	10.7	30.4	10.3	18.3	8.5	17.3	5.7
	Double first cousins	33.0	12.0	13.0	7.3	10.6	6.7	65.8	27.0	32.0	13.2	27.7	12.4	16.9	10.0	15.2	7.1

*Number of items in the test.

†The left-hand numbers represent the number of items in the test for grade 4 and the right-hand numbers, number of items for grade 6.

depression seems clear and consistent in both. It is interesting that the effect is smaller for the mathematics and substitution tests, though the three groups fall into the same order here as for the other tests.

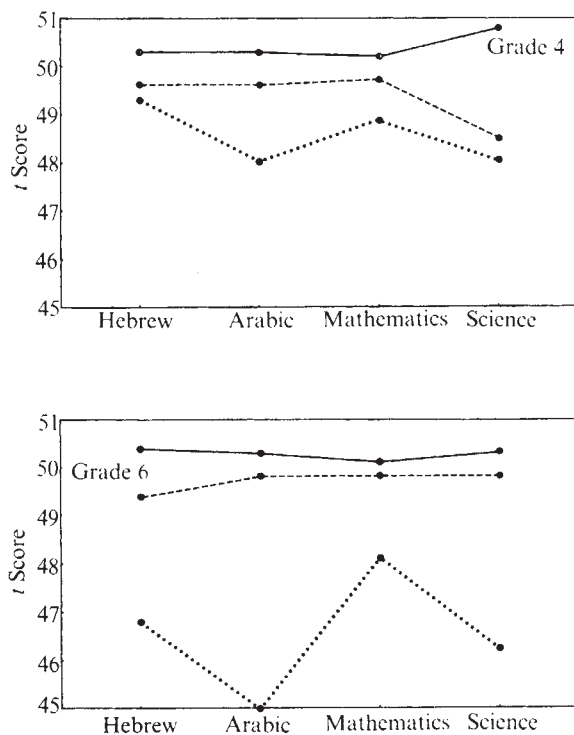


Fig. 2 Standardised means (*t* scores) of achievement tests according to parental consanguinity for grades 4 and 6. —, Not related; ---, cousins; . . . , double cousins.

The inbreeding depression clearly demonstrates the importance of a genetic component in the variance of cognitive performance in this population. This could result either from the general increase in homozygosity that results from inbreeding or from decrease in performance resulting from homozygosity for specific recessive alleles^{7,8}. The higher variance of the double cousin group in some of the tests favours the second interpretation.

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The significance of the nose for certain phenomena of visual perception

BOWER¹ draws attention to the fact that the majority of organisms have evolved in such a way that part of the body (almost invariably the nose) is permanently present in the visual field. This, as he points out, can hardly be accidental and indicates that the organ may have a function in visual perception; subjects lacking a nose may differ from normals on tests of visual capacity. Unfortunately this clinical condition is now rarely encountered and affected individuals may, in any case, develop compensatory techniques, for example, reliance on glimpses of some other part of the body. It may be more profitable to consider the effects of making the nose visible where this is not normally the case. The autokinetic phenomenon may, for example, be a consequence of the fact that in total darkness the nose is invisible and cannot play its customary role as a stable point of reference by which other objects in the visual field are judged stationary or in motion with respect to the observer. What would be the effect of making the nose visible in an otherwise dark visual field. Contrary to Bower, autokinetic movement seems to have persisted.

The autokinetic phenomenon occurs when a subject fixates a point source of light in an otherwise dark field. The light characteristically appears to move, though it is in fact stationary throughout. Recent experimental work has vastly increased our understanding of the parameters governing the extent of this phenomenon, but none of the proposed explanations has survived experimental test. Bower's proposal implicating the nose is *prima facie* appealing but has not been confirmed empirically. What is required is a means of making the nose visible to the subject in an otherwise dark visual field since movement should not then be reported. Two alternative methods suggest themselves. The subject may wear a luminous false nose, or small electric light bulbs may be inserted in his nostrils illuminating the nose from within. Each method has disadvantages. The luminous false nose has a pale greenish-white appearance and does not correspond in size and conformation to the subject's own nose (being usually somewhat larger). The inserted light bulbs involve a degree of physical discomfort but the organ visible is the subject's own, even though translucent pink in colour. Both methods were used in the two experiments to be reported. The

luminous false nose condition presented no special problems of method but in the inserted light bulb condition it was found that because the conformation of the nostril and the thickness of the nasal membrane differ from subject to subject, a greater or lesser area around the nose was sometimes illuminated. To ensure that no additional visual cues were available, the subject was enveloped from the neck down in a voluminous cloth of black, non-reflective material similar (except in colour) to those used by hairdressers.

In Experiment 1 each subject spent three 2-min periods in total darkness fixating the point source and reported the onset and offset of apparent movement if any occurred. Ear-muffs were worn to avoid interference from irrelevant auditory stimuli. The subject was allowed 5 min to adapt to the darkness before beginning his first observation period and rested for 3 min in dim illumination between periods.

Each subject did one period wearing a luminous false nose, one with light bulbs inserted in his nostrils and one control period. A standard moulded rubber artificial nose was used, coated with non-toxic luminous paint. The nostril illuminator consisted of a pair of 6-V, 0.36-W L.E.S. bulbs partially embedded in plastic. These were disinfected between subjects using 5% cetrimide solution. Twelve subjects were tested (all female, average age 18.56 yr). In order to equalise the effects of practice and fatigue the three experimental conditions were presented to two subjects in each of the six possible orders of presentation.

The results obtained are shown in Table 1. Three subjects did not report movement in any condition, results are therefore tabulated for nine subjects only.

Table 1 Incidence of autokinetic movement in Experiment 1

	Illuminated nostril	Control	Luminous nose
Time of onset (s)	38.22	47.44	28.11
Duration (s)	41.67	38.44	55.78

There is no significant difference between the illuminated nostril condition and the control condition, either in time of first onset measured from the beginning of the period ($t=0.25$, not significant), or in duration—that is, total time during which the point of light was in (apparent) motion ($t=0.28$, n.s.). The luminous false nose condition seems to have enhanced the effect (onset, $t=1.65$, n.s.; duration, $t=2.32$, $P<0.05$).

It might be argued that the control condition in Experiment 1 was inadequate, since although the subject was unable to see his nose he was also free of the physical sensations and possible discomfort associated with the experimental conditions (particularly the illuminated nostril condition). In a second experiment each experimental condition was compared directly with a control condition in which the subject wore an ordinary (non-luminous) false nose or in which light bulbs were inserted in the nostrils but were not illuminated. Twenty-four subjects were tested in all four conditions (12 male, 12 female, average age 18.67 yr). The four experimental conditions were presented once each in the 24 possible orders of presentation. Procedure was otherwise as in Experiment 1.

The results obtained are shown in Table 2. No effects of

Table 2 Incidence of autokinetic movement in Experiment 2

	Illuminated nostril	Non-illuminated nostril	Luminous nose	Non-luminous nose
Time of onset (s)	41.33	27.37	25.71	24.58
Duration (s)	49.50	61.50	59.00	61.92

sex were observed. All subjects reported movement in at least two conditions.

The luminous false nose condition did not affect the phenomenon, but the illuminated nostril condition seems to have been associated both with later onset and with shorter duration of apparent movement. Unfortunately, these differences (which would have supported Bower's hypothesis) fail to reach significance by the t test (onset, $t=1.71$, n.s.; duration, $t=1.53$, n.s.).

It may be that the techniques employed were not sufficiently refined. (We experienced some difficulty in persuading subjects unfamiliar with the requirements of strict experimental method to sit in total darkness, wearing ear-muffs, with electric light bulbs inserted in their nostrils—particularly when the bulbs were not actually illuminated). But the project is at an early stage. The results so far obtained while not positively refuting Bower's hypothesis, cannot be said to support it.

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Sampling theory for alleles in a random environment

A MAJOR effort in theoretical population genetics has been aimed at developing statistical tests to distinguish between various explanations of observed patterns of enzyme variation¹. Perhaps the most useful result to emerge from this effort is Ewens' sampling theory of neutral alleles². This theory allows one to use the number and configuration of alleles in a sample to test for departures from neutrality. Watterson³ has derived the analogous sampling distribution for a finite population subject to the joint effects of mutation and symmetric heterotic selection. It differs from the neutral sampling theory, providing a stronger hypothesis-testing situation than has previously been available. In this note I present a third sampling distribution. It applies to an infinite population in which the selective coefficients fluctuate at random through time and space. Quite unexpectedly, the sampling distribution turns out to be identical to that for the neutral model, indicating that agreement of data with Ewens' sampling distribution does not rule out the possibility that natural selection is responsible for the genetic variation in the sample. Furthermore, the properties of random-selection populations at equilibrium are indistinguishable from neutral-allele populations for observations made at a single time point. This result calls into question the claim that agreement of data with predictions of the neutral model argues against natural selection as an important factor for the maintenance of genetic variation.

Work on biologically-motivated models for natural selection in a random environment has shown that, under a wide variety of assumptions, two allele models yield diffusion processes with drift and diffusion coefficients of the form

$$\begin{aligned}\mu(x) &= x(1-x)[a - b(1/2 - x)] \\ \sigma^2(x) &= x^2(1-x)^2\end{aligned}\quad (1)$$

For symmetric models, which will be the only ones considered here, $a = 0$, and

$$\begin{aligned}\mu(x) &= bx(1-x)(1/2 - x) \\ \sigma^2(x) &= x^2(1-x)^2\end{aligned}\quad (2)$$

For example, for a diploid species with additive alleles in a subdivided environment

$$b = 2/[1 - \pi(1-k)] \quad (3)$$

where π is a measure of the patch structure and k is a measure of the correlation between patches⁴. Biological considerations suggest that $b \geq 2$, with larger b values associated with greater environmental heterogeneity. As another example, if the environments are autocorrelated, we can still obtain a diffusion process for the additive, diploid case, with

$$b = \frac{2}{1+\lambda} \quad (4)$$

where λ is a measure of the autocorrelation of the environment⁵. In this instance $1 < b < \infty$, with smaller values of b associated with more positively autocorrelated environments. Other models incorporating dominance and various population structures also give models of the same form as (2), with, in general, $1 < b < \infty$ (ref. 6). The asymptotic density for (2) is a β distribution

$$\Phi_2(x) = \frac{\Gamma(2b-2)}{\Gamma(b-1)^2} [x(1-x)]^{b-2} \quad (5)$$

which always exists since $b > 1$. Two neutral alleles with equal mutation rates also yield a beta distribution of this form which hints of the reason why random environment models behave, asymptotically, like neutral allele models.

The K -allele extension to (2) may be written, using techniques, similar to those in Gillespie⁷, as

$$\begin{aligned} \mu_i(\tilde{X}) &= bx_i(F-x_i) \\ \sigma_i^2(\tilde{X}) &= x_i^2(1+F-2x_i) \quad i = 1, 2, \dots, K-1 \\ \sigma_{ij}(\tilde{X}) &= x_i x_j (F-x_i-x_j) \end{aligned} \quad (6)$$

where $X = (x_1, x_2, \dots, x_{K-1})$, $F = \sum_{i=1}^K x_i^2$, and $x_K = 1 - x_1 - x_2 - \dots - x_{K-1}$. We can solve the three allele case by using the zero probability flux condition given in Kimura⁸ and following the techniques given in his equations (9.5) to (9.7). If we write the asymptotic density as

$$\Phi_3(x_1, x_2) = \exp(\psi(x_1, x_2)) \quad (7)$$

we are led to the system

$$\begin{bmatrix} \sigma_1^2 & \sigma_{12} \\ \sigma_{12} & \sigma_2^2 \end{bmatrix} \begin{bmatrix} \frac{\partial \psi}{\partial x_1} \\ \frac{\partial \psi}{\partial x_2} \end{bmatrix} = \begin{bmatrix} -2\mu_1 - \frac{\partial}{\partial x_1} \sigma_1^2 - \frac{\partial}{\partial x_2} \sigma_{12} \\ -2\mu_2 - \frac{\partial}{\partial x_1} \sigma_{12} - \frac{\partial}{\partial x_2} \sigma_2^2 \end{bmatrix} \quad (8)$$

which yields, after considerable manipulations

$$\begin{aligned} \frac{\partial \psi}{\partial x_1} &= \frac{2b-5}{3} \left[\frac{1}{x_1} - \frac{1}{1-x_1-x_2} \right] \\ \frac{\partial \psi}{\partial x_2} &= \frac{2b-5}{3} \left[\frac{1}{x_2} - \frac{1}{1-x_1-x_2} \right] \end{aligned} \quad (9)$$

Integrating this system gives

$$\Phi_3(x_1, x_2) = \frac{\Gamma(2b-2)}{\Gamma((2b-2)/3)^3} [x_1 x_2 (1-x_1-x_2)]^{(2b-6)/3} \quad (10)$$

This is a Dirichlet distribution and suggests immediately that the form of the asymptotic density for K alleles will be

$$\Phi_K = C \left[\prod_{i=1}^K x_i \right]^{\beta} x_K = 1 - x_1 - x_2 - \dots - x_{K-1} \quad (11)$$

If we substitute (11) into the equation giving the zero probability flux condition:

$$-\mu_i \Phi_K - \frac{1}{2} \frac{\partial}{\partial x_i} [\sigma_i^2 \Phi_K] - \frac{1}{2} \sum_{j \neq i} \frac{\partial}{\partial x_j} [\sigma_{ij} \Phi_K] = 0 \quad (12)$$

we discover that the appropriate value of β is

$$\beta = \frac{2(b-1)}{K} - 1 \quad (13)$$

Thus the asymptotic density for the system (6) is the Dirichlet distribution

$$\Phi_K(X) = \frac{\Gamma(2b-2)}{\Gamma((2b-2)/K)^K} \left[\prod_{i=1}^K x_i \right]^{\frac{2b-2}{K} - 1} \quad (14)$$

This is the same form as the asymptotic density for the K -allele neutral model (see refs 7, 9, 14).

The tractability of the sampling distribution of neutral alleles rests on the assumption that the underlying process has an infinite number of possible allelic states. The natural analogue in our case would be to consider the behaviour of Φ_K as $K \rightarrow \infty$. By this procedure we do not mean to imply that there are, in any real population, an infinite number of alleles, rather we view the $K = \infty$ case as an approximation to the behaviour of a large number of alleles. The behaviour of both Φ_K and samples from Φ_K are well known, the most powerful results coming from recent work by Kingman¹⁰.

As $K \rightarrow \infty$, the limiting form of Φ_K degenerates, but the distribution of the order statistics

$$x_{(1)} \geq x_{(2)} \geq x_{(3)} \geq \dots \quad (15)$$

converges to the Poisson-Dirichlet limit. Kingman¹¹ has recently proved the remarkable theorem—"a sequence of populations has the Ewens sampling property if and only if it has the Poisson-Dirichlet limit."

Thus the sampling distribution for alleles subjecting to selection in a fluctuating environment according to equation (6) has the same sampling distribution as the neutral allele model. The fact that the Dirichlet distribution yields Ewens' sampling distribution as $K \rightarrow \infty$ was apparently shown first by Frank Stewart (unpublished results). Watterson¹² showed independently, that the order statistics of the Dirichlet distribution converge to a limit, and obtained the joint density for the first r order statistics.

For our parameterisation we can write down certain sampling properties immediately using the results of Ewens². The probability that a sample of size $2n$ contains k alleles is

$$Pr[k|2n] = \frac{(2b-2)^k}{L(2b-2)} \quad (16)$$

The probability that a sample of size $2n$ with k allelic types contains n_i alleles of type i is

$$Pr[n_1, n_2, \dots, n_k | k, 2n] = \frac{2n!}{k! 1_{n_1} n_1 \dots n_k} \quad (17)$$

where, in (16) and (17)

$$L(y) = y(y-1)(y+2) \dots (y+2n-1) \\ = 1_1 y - 1_2 y^2 + \dots + 1_{2n} y^{2n}$$

Similarly, we can write down the expected homozygosity as $EF = 1/(2b-1)$, and the frequency spectrum, as used by Yamazaki and Maruyama¹³ as $x^{2b-2} / (1-x)^{2b-2}$.

Examination of these results shows that heterozygosity increases with b . From equations (3) and (4) we are led to the observation that increasing the environmental heterogeneity will increase the heterozygosity, while increasing the autocorrelation will decrease the heterozygosity.

It was quite unexpected that the infinite-allele random-environment model should behave in a manner completely analogous to the neutral-allele model. Ewens's² original derivation of the sampling distribution used intuition concerning the behaviour of unique alleles which appear in the population through mutation, continue to segregate for a time, and are then lost. Clearly this line of reasoning does not immediately carry over to the present model because of its inaccessible boundaries. Also, in the neutral model the frequency of a single allele is a Markovian variable whose behaviour can be studied in isolation by lumping the frequencies of all other alleles. This is not the case for the random environment alleles whose individual behaviour depend on F and cannot be studied without some knowledge of the actual frequencies of the other alleles.

The assumption of complete symmetry given in equation (6) is probably unnecessary. In the two allele case even asymmetric models give asymptotic β distributions. If this carries over to K -alleles, then the results of Kingman¹⁰ indicate that even asymmetric Dirichlet distributions can have the Poisson-Dirichlet limit providing certain conditions are met. The common observation that there are 'too many' rare alleles in many samples from nature¹⁴ can be accounted for by these asymmetries.

The present model is limited in that it does not include finite-population effects. Whether this will seriously affect the behaviour of alleles which are frequent enough to appear in samples is not known.

Finally, since many of the tests for neutrality are based on the asymptotic behaviour of the infinite-allele neutral model, the present results indicate that none of these tests can distinguish neutrality from random-environment behaviour. Included among these tests would be those of Ewens² Yamazaki and Maruyama¹³, Johnson and Feldman¹⁵ and Nei *et al.*¹⁶.

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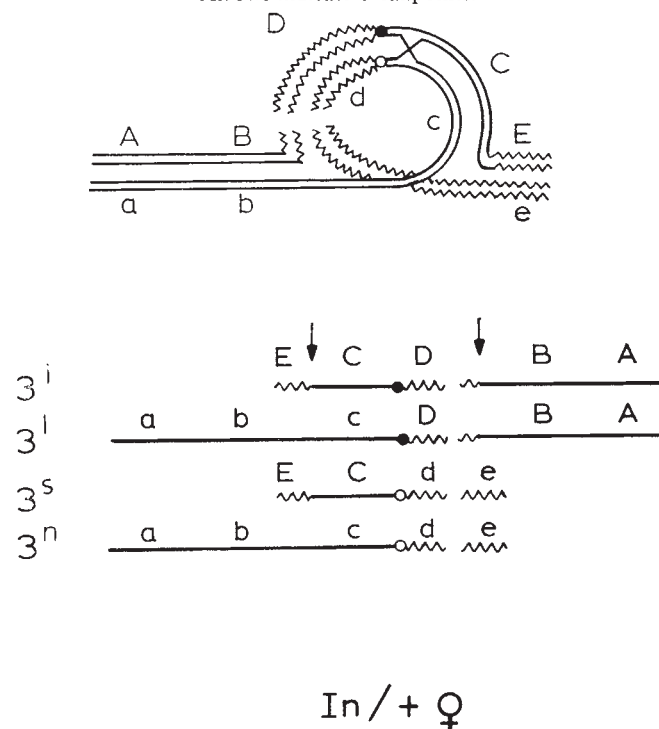
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Synthesis of compound chromosomes from a pericentric inversion in the onion fly *Hylemya antiqua*

STRUCTURAL chromosome mutations such as reciprocal translocations^{1,2}, pericentric inversions³ and compound chromosomes^{4,5} have been considered for the control of insect pests. Their exploitation is based on the capacity to reduce the fertility of a population, and they might be used to introduce a gene into a natural population to render the pest less harmful. This possibility arises from the fact that some structural chromosome mutations confer higher sterility in crosses to wild type, or in the hybrids from such crosses, than in matings within the mutant strain. This property is particularly evident with compound chromosomes which have homologous arms attached to common centromeres rather than to different centromeres. This causes intersterility with wild-type individuals, because all the hybrids carry chromosome duplications and deficiencies. In contrast to studies⁶⁻⁷ in which compound chromosomes were isolated starting from two almost whole arm reciprocal translocations. I have used a pericentric inversion in the onion fly, *Hylemya antiqua*.

Four different types of chromosomes were produced if crossing over occurred anywhere within the loop of a pericentric inversion heterozygote⁸. Figure 1 shows how these types were discriminated on the basis of total length and arm ratio. The normal (3^n) and inverted (3^i) chromosomes are balanced. The other two crossover chromosomes (3^s and 3^l) both carry duplications and deficiencies and are considered as compound chromosomes, although the breakpoints are not in the immediate vicinity of the centromere. If both sexes were chiasmate, a compound chromosome strain could be made from crosses between inversion heterozygous parents. Because only female onion flies are chiasmate, I looked for males carrying a chromosomal rearrangement which gave products which would be complementary to one of the compound chromosomes produced by the inversion female (Fig. 2). Crosses between inversion females

Fig. 1 Diagram of a pericentric inversion heterozygote and the possible products. 3^i and 3^n are the parental combinations and 3^l and 3^s the results of crossing over in the inversion loop. Arrows indicate breakpoints.



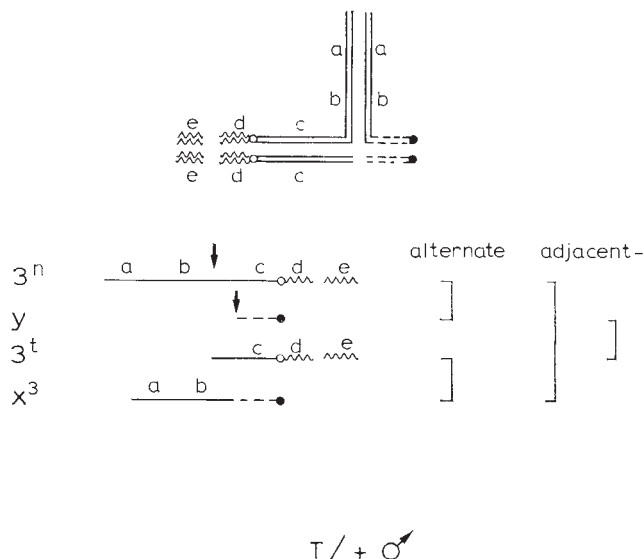


Fig. 2 Diagram of the translocation heterozygote (male) and the two orientation types.

and translocation males produced some progeny with the compound type of chromosome, consisting of a large re-combinant chromosome (3^l) from the female and a short complementary translocated chromosome (3^i) from the male. It was intended that subsequent backcrossing of such males with inversion females would give a compound chromosome strain carrying 3^i and 3^s .

Cytological analysis (Fig. 3) of an X-linked translocation⁹ showed that one of the translocated chromosomes (3^i) resembled the short chromosome (3^s) from the inversion. Four different chromosomes were involved in the translocation, which is a simple one with a breakpoint in the telomeric area of the X chromosome (Figs 2 and 3). Four different types of gametes were produced in equal frequency and 16 different embryonic karyotypes were assumed to occur when the translocation males are crossed to inversion females. As Table 1 shows, five karyotypes were deficient for segment ab (about the distal two-thirds) of the long arm of chromosome 3. Indeed these types were not observed in the larval stage and died as embryos.

Eleven different karyotypes were expected to be viable in the larval stage, but observations on mitotic preparations with somatic pairing revealed only eight types. Of the three missing categories I could not explain the absence of $3^i3^sX^3X$ larvae which are no more duplicated than, for example, the viable 3^i3^sXY type. The $3^i3^sX^3X$ type is tetrasomic for segment ab and therefore probably too duplicated to survive. The $3^i3^sX^3X$ larval karyotype is probably indistinguishable

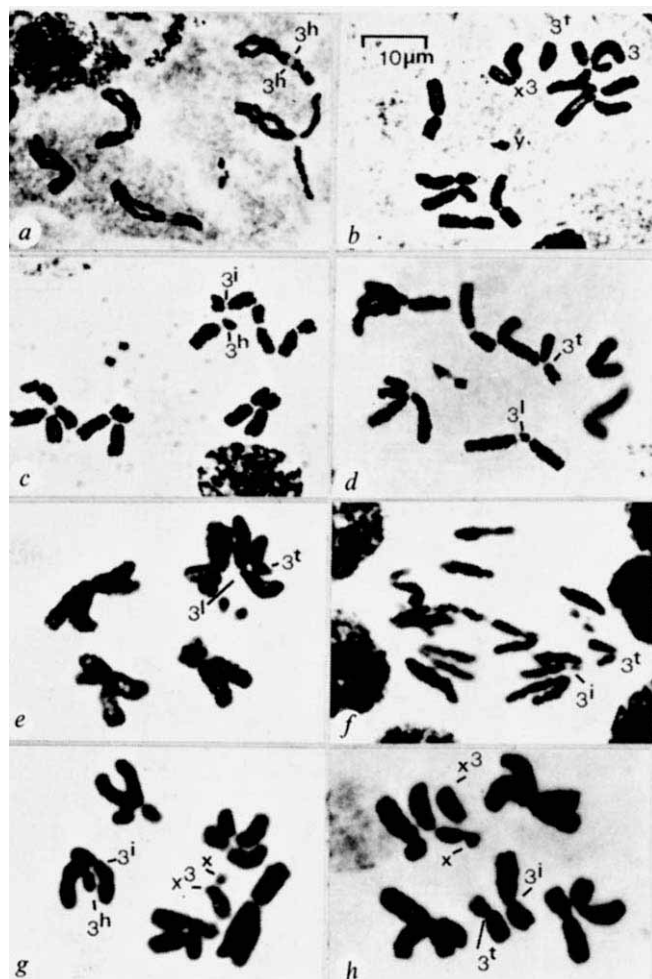


Fig. 3 a, Normal karyotype with two 3^n chromosomes, prophase in larva ($2n = 12$). b, Translocation heterozygote $3^i3^sX^3Y$ (male), metaphase in larva. c, Pericentric inversion heterozygote 3^i3^n , metaphase in larva. d and e, Larval compound type 3^i3^l , 3^i from $T/+$ ♂ and complementary 3^l from $In/+$ ♀. Mitotic metaphase. f, Compound type, mitotic anaphase. Note constrictions in 3^l . g, $3^i3^sX^3X$, mitotic metaphase in larva. h, $3^i3^sX^3X$, mitotic metaphase in larva.

from the translocation heterozygote ($3^i3^sX^3X$). Out of 40 larvae analysed, three were of the male compound type (3^i3^lXY) (Fig. 3d, e and f). Unfortunately no compound karyotype was observed among adult males (30) and therefore no compound strain was isolated. Probably a small terminal deficiency (segment e), distal to the breakpoint in the short arm of chromosome 3 (one-tenth of the chromosome), caused inviability. This was unfortunate because the

Table 1 Gametic combinations after mating of $In/+$ females and $T/+$ males

In/+ ♀	T/+ ♂	Alternate										Adjacent—1									
		3 ⁿ X ³					3 ⁿ Y					3 ^t Y					3 ⁿ X ³				
3 ⁿ		e	d	c	b	a	e	d	c	b	a	e	d	c	b	Δ	e	d	c	bb	aa
		e	d	c	b	a	e	d	c	b	a	e	d	c	b	a	e	d	c	b	a
3 ^l		e	d	c	b	a	e	d	c	b	a	e	d	c	b	a	e	d	c	bb	aa
		D	c	Bb	Aa		D	c	Bb	Aa		D	c	Bb	Aa		D	c	Bb	Aa	
3 ^s		e	d	c	b	aΔ	e	d	c	b	aΔ	e	d	c		Δ	e	d	c	bb	aa
		Ee	d	C			Ee	d	C			Ee	d	C			Ee	d	C		
3 ⁱ		e	d	c	b	a	e	d	c	b	a	e	d	c	b	a	e	d	c	bb	aa
		E	D	C	B	A	E	D	C	B	A	E	D	C	B	A	E	D	C	B	A
				♀					♂					♂					♀		

The letter combinations indicate the presence of duplications and/or deficiencies. The compound type is indicated by an asterisk. The Δ types are expected to die in the embryonic stage. Y is the male determining factor. Photomicrographs of some karyotypes (larvae) are shown in Fig. 3.

presence of a small duplication for a tiny interstitial segment, located between the long arm breakpoints of two rearrangements, usually has little influence^{8,9,10}.

To avoid the problem of trying to obtain a translocation which is more exactly complementary to the crossover products of the inversion, another suggested method for the production of a compound chromosome strain is the irradiation of inversion heterozygous males to stimulate crossing over in the loop¹¹. Then the recombinant chromosomes 3⁺ and 3⁻ could be generated through both sexes. The usefulness of the pericentric inversion would be much reduced if the distances of both breakpoints to the centromere are too large. Otherwise crossing over is to be expected between the two compound chromosomes, thus producing normal and inverted chromosomes again and hence intersterility with the wild type would be incomplete. This would prevent use of the system to replace completely a wild population by a harmless form. But in a species with achiasmatic males, this sex from such a compound chromosome strain would be fully sterile in matings to wild type. Thus such a strain could be used as a source of material for a sterile male release programme.

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Regulation of dimorphism in the pathogenic fungus *Histoplasma capsulatum*

Histoplasma capsulatum is a dimorphic pathogenic fungus which is the causative agent of the disease histoplasmosis. The disease is worldwide in occurrence and approximately 40 million people have been infected in the USA¹. In soil the fungus is filamentous, but in the infected host it exists as a budding yeast and it is believed that the phase transition is important in the pathogenicity. In the laboratory, the mycelial form can convert to the yeast phase when the temperature of incubation is shifted from room temperature (25 °C) to body temperature (37 °C). In this report we show that the elevated temperature initiates a series of reactions leading to changes in the intracellular level of 3', 5'-cyclic adenosine monophosphate (cyclic AMP); the shifts in intracellular cyclic AMP levels are an important determinant of the morphological phase of the organism and therefore of its disease producing potential.

Earlier studies²⁻⁹ have already shown that the natural signal of temperature is not by itself critical to cause the phase transition. Rather, temperature seemed to affect the level of sulphhydryl groups or the redox potential, which in turn determined the phase of the organism. For example, added sulphhydryl, usually in the form of cysteine, could force mycelia into the yeast phase even at 25 °C (ref. 3). Moreover, Rippon showed¹⁰ that a low redox potential imposed even in the absence of added cysteine, stimulated the conversion of mycelia to yeast at 25 °C, whereas yeast converted to mycelia when the medium had a higher redox potential.

In attempting to analyse further the role of sulphhydryl groups, we started from the observations of Garrison that cysteine and cystine were actively transported into yeast by an ATP-dependent

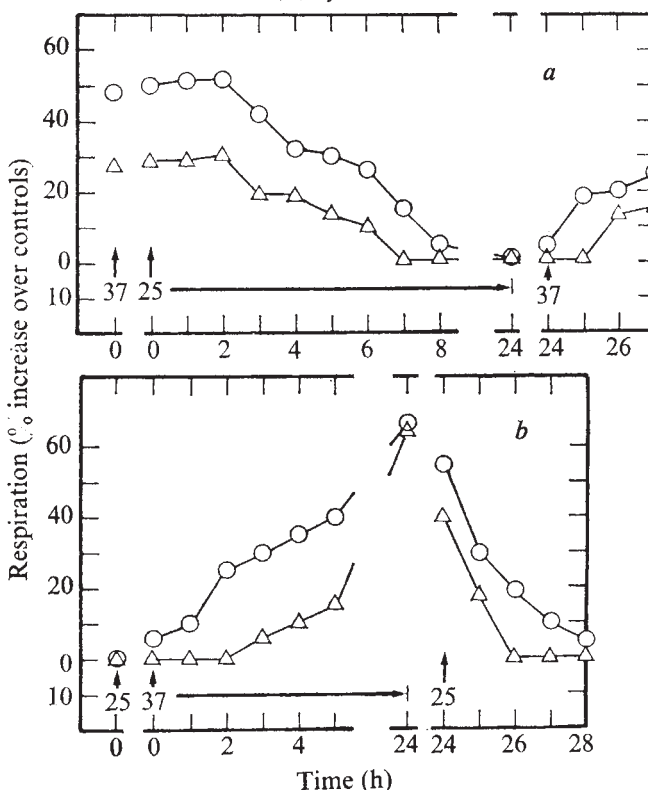
permease, and that they stimulated fungal respiration by entering the Krebs cycle as pyruvic acid¹¹. Garrison also found that the effects on respiration were a good measure of the active uptake of these compounds.

We found that yeast incubated at 37 °C had a higher level of respiration than mycelia incubated at 25 °C (6.6 and 2.8 nmol O₂ per min per mg dry weight, respectively). When yeast cells were brought to 25 °C, however, respiration immediately fell to the level characteristic of mycelia (2.2 nmol). Similarly, when mycelia were incubated at 37 °C, there was an immediate increase to the level observed with yeast (6.5 nmol). We concluded that the baseline level of respiration in the two phases of *H. capsulatum* was a direct response to temperature of incubation and reflected only the QO₂ of mitochondrial function.

The addition of cysteine or cystine to the medium stimulated the respiration of yeast but not of mycelia. When yeast cells were switched from 37 to 25 °C, respiration fell immediately and the stimulus to respiration of cysteine and cystine gradually diminished to zero over 8 h (Fig. 1). After 24 h of incubation at 25 °C, the temperature was abruptly brought back to 37 °C. Baseline respiration increased immediately and there was a rapid return of the stimulus to respiration by added cysteine (within 5 min) and a later return with added cystine (Fig. 1). When the temperature was switched to 37 °C in the presence of 1 mg ml⁻¹ cycloheximide and 1 mg ml⁻¹ chloramphenicol, the cysteine-induced change in respiration still occurred. At these concentrations of antibiotics, protein synthesis was less than 20% of control cells¹².

Mycelia incubated at 25 °C did not respond to cysteine or cystine, but when the cells were switched to 37 °C they became responsive to these amino acids within 5 min (Fig. 1). The rapid changes in uptake of cysteine and cystine, even in

Fig. 1 Effect of cysteine (○) and cystine (△). *H. capsulatum* (Down's strain mating type [-]) was grown in 2% glucose 1% yeast extract (GYE) in a New Brunswick rotary shaker. An inoculum of 10 mg dry weight of mycelia per ml was incubated at 25 °C, and an inoculum of 5 × 10⁶ yeast cells per ml was incubated at 37 °C. Oxygen consumption was measured using a Clark electrode. The dry weights were determined by filtering 5 ml culture through 0.22-μm Millipore filters and drying the filters at 60 °C to constant weight. Daily fresh solutions of cysteine (pH 6.4) and cystine were prepared. The final concentrations were respectively 10 mM and 21 μM. Rate of growth of the two phases as determined by dry weights was the same. *a*, Yeast cells; *b*, mycelial cells.



cycloheximide-chloramphenicol-treated cells, suggested that this process did not require new protein synthesis and that transport protein(s) may be present on cell membranes of both phases of *H. capsulatum*, but only active at 37 °C.

If the action of temperature is to change membrane redox potential, and this can be facilitated by activating a permease for cysteine uptake, fungal dimorphism may be controlled by some critical sulphhydryl groups, or by a general change in reducing state. To test this, *p*-chloromercuriphenisulphonic acid (PCMS), a sulphhydryl-blocking agent, and dithiothreitol (DTT), a sulphhydryl protector, were tested for their effects on the phase transition. Mycelia were exposed to 100 µM PCMS for different lengths of time (5 min to 2 weeks) and were then washed twice, resuspended in fresh medium without PCMS and plated on GYE agar plates at 37 °C. The cells grew continuously after this treatment but never reverted to the yeast form (Fig. 2). PCMS (100 µM) also accelerated the transition of yeast to mycelia at 25 °C. After exposure to PCMS and incubation at 25 °C, yeast transformed to mycelia in 5–6 d instead of the usual 12–14 d. Even yeast incubated at 37 °C began to transform to mycelia in the presence of PCMS, but reverted to normal yeast when PCMS was washed out.

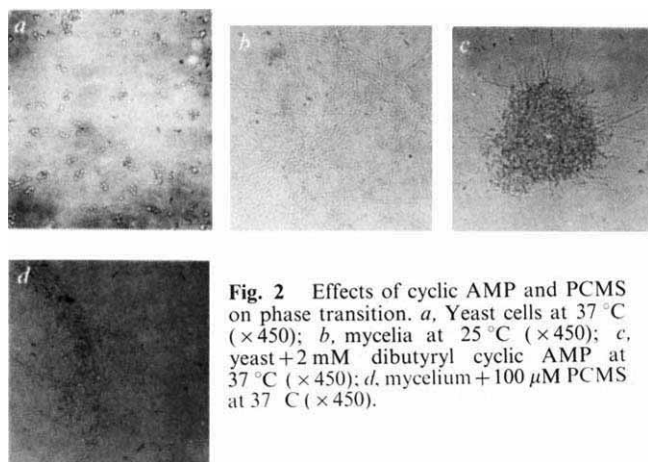


Fig. 2 Effects of cyclic AMP and PCMS on phase transition. *a*, Yeast cells at 37 °C ($\times 450$); *b*, mycelia at 25 °C ($\times 450$); *c*, yeast + 2 mM dibutyryl cyclic AMP at 37 °C ($\times 450$); *d*, mycelium + 100 µM PCMS at 37 °C ($\times 450$).

Opposite effects were found when cells were treated with DTT. For example, when yeast were incubated at 25 °C in the presence of 5 mM DTT, transformation to mycelia did not occur (although in this case the transition to mycelia still occurred when DTT was removed from the medium). Neither PCMS nor DTT were toxic to either phase of *H. capsulatum* at the concentrations used.

The effects of PCMS and DTT argue that sulphhydryl groups, most likely at the surface of the cell, are critical for determining the phase of *H. capsulatum*. The irreversibility of the PCMS effect was unexpected, but also suggested that some surface feature was critical. The next question was how critical sulphhydryl groups, especially at the membrane of the organism, could regulate a complex transition of yeast to mycelium. One possible explanation was that this phenomenon was similar to the effects of many hormones on eukaryotic cells, which often begin with a membrane change that modifies the intracellular level of a cyclic nucleotide.

In *H. capsulatum*, we found that the level of cyclic AMP was about five times higher in the mycelial phase than in the yeast phase. Cyclic AMP was assayed in yeast and mycelia by the radioimmunoassay of Steiner *et al.*¹³ or with the Amersham-Searle cyclic AMP assay kit. The cells were collected by filtration, washed three times in ice-cold water, suspended in either 5% TCA, 0.1 N HCl or 0.05 M Tris-HCl buffer, pH 7.5, containing 1 mM 3-isobutyl-1-methylxanthine (Aldrich Chemicals). Glass beads (diameter 0.45–0.50 mm) were added to the suspension and the cells were disrupted at 4,000 r.p.m. for 3 min in a B. Braun Cell Homogeniser MSK, cooled with liquid CO₂. The resulting homogenates were centrifuged and an aliquot of the supernatant was lyophilised directly, or, in the case of cells homogenised in TCA, after extraction in ether. The dried material was resuspended in the appropriate buffer, and the cyclic AMP content determined. The recovery of added cyclic AMP in controls

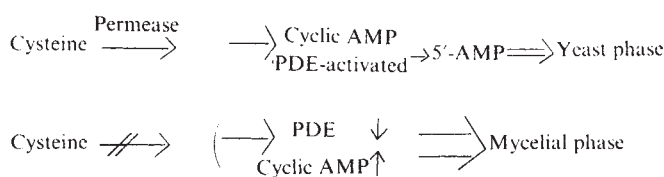


Fig. 3 Working hypothesis for role of cysteine and cystine in morphogenesis of *H. capsulatum*.

was 80–90%. The supernatant of cells homogenised in Tris buffer containing methylxanthine and precipitates of HCl and TCA-extracted cells redissolved in 1 N NaOH were assayed for protein according to Lowry *et al.*¹⁴. The average of three determinations showed that mycelia contained 46.9 and yeast 9.8 pmol cyclic AMP per mg protein. In addition, 2 mM dibutyryl cyclic AMP or 5 mM theophylline added to yeast cultures resulted in the conversion of yeast to mycelia, even at 37 °C, the temperature characteristic for the yeast phase (Fig. 2).

Thus, although the role of cysteine and cystine in morphogenesis of *H. capsulatum* is not understood, it seems likely that they satisfy a requirement for critical sulphhydryl groups that then determine the level of intracellular cyclic AMP. We do not know how this might occur, but possibly, as in *Escherichia coli*, the activity of cyclic AMP-phosphodiesterase (cyclic AMP-PDE) requires the presence of a reducing substance¹⁵. The redox potential, governed by sulphhydryl groups, could then have a regulatory role in the level of cyclic AMP, which in turn would control morphogenesis. Therefore, we suggest as a working hypothesis the schema of Fig. 3. At 37 °C the cells are able to bring cysteine to critical membrane sites. There it would activate cyclic AMP-PDE, thereby lowering intracellular cyclic AMP and maintaining the yeast phase. When the temperature is switched to 25 °C, transport of cysteine decreases and cyclic AMP-PDE activity falls. This results in an increase in cyclic AMP levels and a shift to the mycelial phase.

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The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces

ALTHOUGH the ecological role of bacterial chemotaxis has been the object of considerable speculation, little is known of its role in nature¹. Moreover, chemotaxis as a factor in the interaction between the mammalian host and its indigenous or pathogenic microflora remains unexplored. Studies with *Vibrio cholerae* and other intestinal pathogens have shown that bacterial association with the mucosa is influenced by bacterial motility, adhesion to brush border membranes, the presence of an indigenous flora and by the inhibitory effects of local antibodies

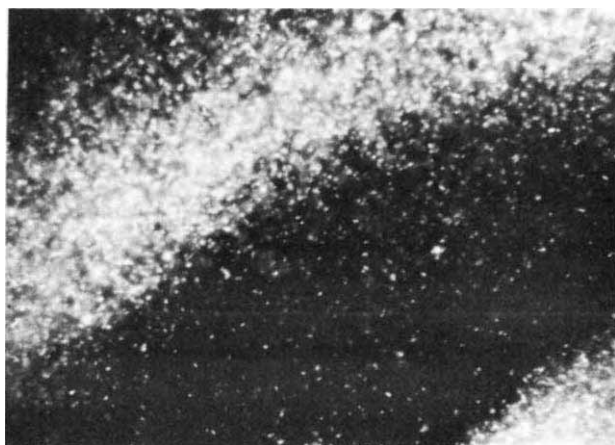


Fig. 1 A chemotactic strain of *S. typhimurium* forming a receding band at some distance from the mucosal surface. The bright triangular area in the lower right corner is the surface of the mucus gel overlaying the villi. The band had originally formed within the mucus gel and some bacteria have been retained there. The diffuse light within the band emanates from bacteria outside the focal plane of the microscope. The diffuse light in the mucus is due to light scattering by the gel itself. Darkfield ($\times 110$).

(summarised in refs 2, 3). We have shown previously that a pepsin digest of rabbit intestinal mucosa (PMS) inhibits the association of cholera vibrios and *Salmonella enteritidis* with rabbit intestine, and speculated that this extract was inhibitory because it neutralised the adhesion of the bacteria to a hypothetical receptor in the mucus gel³. We report here that PMS neutralises a positive chemotactic response of several bacterial species to the mucosa and in that way reduces bacterial association with intestinal tissue. Chemotaxis thus seems to be one of several mechanisms controlling interactions of bacteria with mucosal surfaces.

Observation under a phase-contrast or darkfield microscope of pieces of mouse or rabbit small intestine immersed in a suspension of *V. cholerae* revealed that very high concentrations of vibrios aggregated at the villous surface of this tissue in approximately 1 min. Three non-chemotactic vibrio mutants were selected using a modification of the method of Aswad and Koshland⁴ and differed from the parent strain in that they did not accumulate at the surface of intestinal tissues, but remained randomly distributed throughout the suspending buffer. Similarly rapid attraction to mucosal tissue was shown by *Escherichia coli* (AW 405) and *Salmonella typhimurium* (SL 1634), but not by their non-chemotactic mutants (cheB 590 and SL 2538, respectively). None of the mutants used in this study differed from their respective parents in the rate of motility as determined by direct microscopic observation.

After chemotactic strains of the three bacterial species had accumulated on the surface of intestinal tissues for a period of approximately 2–5 min, the bacterial accumulation began to move away to form a clearly visible band of motile bacteria some distance from the tissue surface (Fig. 1). The distance of separation increased with time until the band eventually reached

the edge of the coverslip. No such bands were formed by any of the non-chemotactic mutants. The formation of these bands may be caused by the local consumption of oxygen at the tissue surface or by the generation of metabolites with negative chemotactic activity by the tissue and/or by the bacteria. Such negative chemotactic gradients would compete with the positive chemotactic gradient generated by the tissue. Accordingly, the bacteria would be expected to accumulate in an area defined by the interaction of opposing gradients. Similar banding phenomena in broth or agar cultures of various bacteria have been described by others^{5–7}. Whatever the actual mechanisms may be, our direct microscopic observations indicate that strikingly effective chemotactic attraction and repulsion of a variety of bacteria can occur at the mucosal surface.

Agar blocks infused with an ultrafiltrate of PMS could be substituted for the mucosal tissue slices. Such blocks attracted *E. coli*, vibrios and salmonellae to their surfaces, indicating that PMS contained one or several attractants. Agar blocks soaked in buffer had no effect. Moreover, the addition of PMS to the bacterial suspension reduced or prevented the chemotactic attraction of vibrios to rabbit mucosal tissue in the microscopic assay. By analogy with other chemotactic systems⁷ it seems likely that the attractants in PMS saturated those chemotactic receptors of bacteria which normally reacted with the attractants emanating from the mucosa, thereby blocking the reactivity of the bacteria. These interactions were also demonstrated with Adler's capillary test for chemotactic activity⁸. Cholera vibrios were attracted into capillaries containing dilutions either of PMS or of a soluble fraction obtained by centrifuging freshly prepared scrapings from the mucosa of rabbit small intestine (Muc. Sn.) (Table 1). The latter preparation presumably contained the chemotactic mediators of mucosal tissue in relatively unaltered form. When PMS was added to the bacterial suspension as well as to the capillaries, no chemotactic gradient could form and no migration into the capillaries occurred. Similarly, the presence of PMS in the vibrio suspension considerably reduced the entry of vibrios into capillaries containing Muc. Sn. (Table 1), suggesting that at least some of the attractants in PMS and Muc. Sn. were similar. No response in the capillary test was observed with the non-chemotactic mutant vibrios.

The effect of chemotaxis on the association of vibrios with intact slices of rabbit ileal tissue was determined as described before³, with the modification that the tissues were incubated in buffer containing 5×10^5 cells per ml of each of the chemotactic parent strain plus one non-chemotactic mutant. The proportions of mutant to parent strains that remained on the washed tissues were determined by preparing agar plate cultures and subsequently transferring a number of individual colonies to semisolid tryptone agar⁶. The proportion of non-chemotactic mutants associated with the tissue slices was 3.0 to 3.8 times lower than their proportion in the suspending fluid, indicating that the mutants had a significantly lower chance of associating with the mucosal tissue than the parent strain (Table 2). PMS in the suspending medium had no effect on the association of the non-chemotactic mutants with tissue slices. In contrast, PMS in the suspending medium reduced the association of the parent strain with mucosal slices by a

Table 1 Chemotactic attraction of *Vibrio cholerae* into capillary tubes and its inhibition by a preparation derived from intestinal mucosa

Content of capillary	Buffer	50% PMS*	5% PMS*	50% PMS*	Muc. Sn.† 2%	Muc. Sn.† 2%	Muc. Sn.† 2%
Content of bacterial suspension	Buffer	Buffer	Buffer	50% PMS*	Buffer	50% PMS*	5% PMS*
No. of bacteria in capillary‡ ($\times 10^{-4}$)	3.5§	142	51	4.0	40	8.5	9.5
	7.0§	97	51	7.0	34	14	12

* Ultrafiltrate of pepsin digested rabbit mucosal scrapings.

† Supernate of centrifuged, freshly prepared rabbit mucosal scrapings.

‡ Capillaries of 0.27 mm diameter, filled with 5 μ l Tris buffer (pH 7.4) containing the materials indicated, were dipped for 60 min into vibrio suspensions. The number of vibrios which had invaded the previously sterile capillaries were then counted in a Petroff-Hausser chamber.

§ Duplicate determinations.

Table 2 Reduced invasion of mucosal tissue by non-chemotactic mutants of *Vibrio cholerae*

Parent strain plus mutant no.*	Fraction of non-chemotactic vibrios in suspending fluid*	Fraction of non-chemotactic vibrios on washed mucosa†	Factor: fluid/mucosa
8	41/93 (44.1%)	23/155 (14.8%)	3.0
6	39/73 (53.4%)	22/155 (14.2%)	3.8
31	61/137 (44.5%)	23/155 (14.8%)	3.0

* Slices of rabbit mucosal tissue were incubated without shaking for 8 min in a suspension of vibrios containing approximately equal concentrations of the chemotactic parent strain plus one non-chemotactic mutant as indicated.

† Based on counts of 31 colonies each cultured from 5 mucosal tissue slices.

factor of two to four³. Consequently, when the chemotactic attraction of the parent strain towards the mucosa was blocked by PMS, its ability to associate with the mucosa was reduced to a level similar to that of the non-chemotactic mutants.

Results obtained in three different experimental systems thus indicate that chemotactic mechanisms can promote as well as inhibit the initial invasion of mucosal surfaces by bacteria. Attractive and possibly repellent substances produced by the tissue are obviously important factors in these processes, but chemotactically active substances such as PMS in the medium bathing the mucosal surface seem to be equally potent modifiers of bacterial invasion. It is tempting to speculate about the *in vivo* role of chemotaxis in the interaction between bacteria and their mammalian host. For example, in the initial stages of infection an intestinal pathogen is rapidly propelled along the small intestine in a bolus of digesta, and the duration of its contact with any given area of the mucosal surface may not be longer than a few seconds. The ability of chemotactically attracted bacteria to quickly approach and penetrate the mucus gel may well be of even greater importance in that situation than in the invasion of the statically incubated tissues studied in the present experiments. Indeed, Chet and Mitchell¹ quote data showing rapid chemotactic movement of bacteria across and against fluid flow planes in flowing streams, that is, in conditions which kinetically resemble those of the small intestine. Furthermore, intestinal contents almost certainly contain chemotactically active substances which may either promote or inhibit chemotactic attraction of bacteria into the mucosa. Such a phenomenon may relate to certain aspects of the long-recognised relationship between diet and susceptibility to infection, a phenomenon for which exact mechanisms are still to be delineated⁹. It is also possible that chemotactically active substances added to the diet may exert a prophylactic effect against infections such as traveller's diarrhoea. The association of indigenous bacterial flora with the mucus layer of the large intestine may also be governed in part by chemotactic mechanisms.

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Helical shape and wall synthesis in a bacterium

THE main skeletal components of most bacterial cell walls are complex polymers called peptidoglycans¹. The primary chemical structures of these and other wall components are well understood but little is known about the arrangement of the molecules in the wall or how new wall material is incorporated during growth¹. We report here that several factors cause cells of *Bacillus subtilis*, normally straight rods (Fig. 1*b–e*), to assume a regular helical shape. We suggest a model for the structure and synthesis of Gram positive cell walls which could explain these observations. Explanations suggested by Mendelson² for an apparently different type of helical growth in *B. subtilis* do not account for our results.

In the model which most satisfactorily explained his observations, Mendelson² suggested that the pattern of wall growth in his mutant, and perhaps other strains, results in the rotation of the two ends of a cell relative to one another. When, in his strain, both ends of a cell filament remain attached to the old spore case this rotation is prevented and the resulting torque is relieved by the whole filament distorting to a helix.

We have observed that helices predominate among the abnormally-shaped cell filaments that occur in cultures of *B. subtilis* in the following conditions (*f* = approximate frequency of helical filaments in the populations).

(1) In medium supplemented with the local anaesthetics chlorpromazine (3×10^{-5} to 4×10^{-5} M; 37 °C) or methochlorpromazine (5×10^{-5} to 6×10^{-5} M; 37 °C); *f* = 50%.

(2) In medium containing penicillin G (0.1–0.25 U ml⁻¹; 30 °C); *f* = 1% (Fig. 1*b–e*).

(3) Certain mutants of strain 168 (ref. 3) resistant to the detergent Triton X-100 (unpublished work) grow predominantly in a helical form, especially near the end of exponential growth. *f* > 95% (Fig. 1*a* and *f*).

(4) In cultures restarting growth after subculturing from stationary phase. *f* < 1%.

(For (1) and (2) exponentially growing cells were subcultured, to an initial density of 2×10^6 ml⁻¹, into media containing the drugs. Strain 168*ind* (ref. 3) and Penassy broth (Difco) were used in (2), (3) and (4) but wild-type Marburg strain and nutrient broth (Difco) in (1).)

Our bacteria were not grown from spores, did not appear as closed loops (Fig. 1) and were grown in liquid media. Therefore, unlike Mendelson's example, anchorage of cell ends could not have contributed to their morphogenesis. In addition, the helices we observed were usually much more tightly coiled (Fig. 1).

Mendelson considered it improbable that helical shape could result from unequal growth rates at different points around the cell circumference because of the complexity of the necessary growth pattern². This argument also applies to our observations — perhaps even more so because of the variety of circumstances involved.

Highton and Hobbs¹ described penicillin-induced helical growth, very similar to that reported here, in a penicillin-resistant mutant of *B. licheniformis*. They found abnormal wall thickening at the inside curvatures of bent cells. Similar thicken-

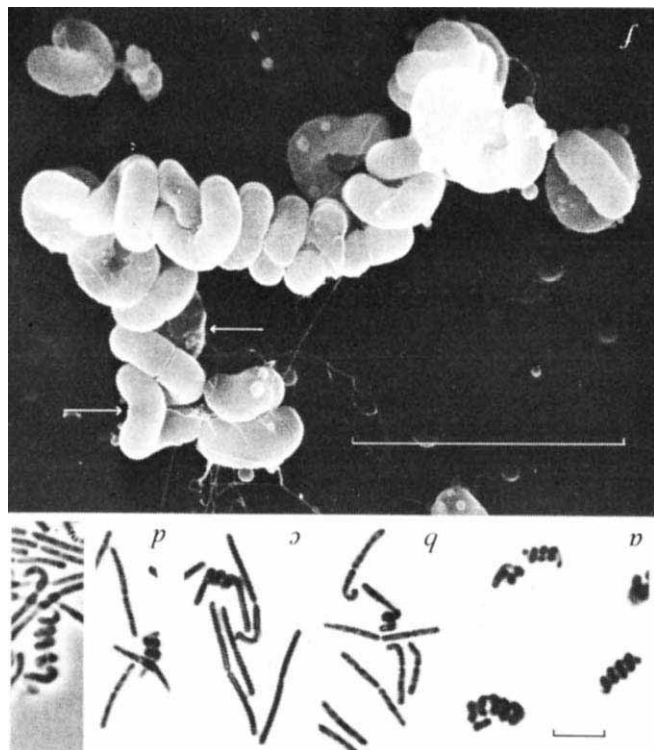


Fig. 1 *a* and *f*, Triton-resistant mutant (TR61) grown in Penassay broth. Arrows in *f* show points of change in direction of helix; *b-e*, normal (straight) and helical forms of strain 168ind⁻ grown in Penassay broth containing Penicillin G (0.25 U ml⁻¹); *a-e*, light micrographs of cells fixed in 1% formalin and dried on to slides; *a-d*, stained with crystal violet; *e*, unstained, and phase contrast illumination; *f*, critical point dried bacteria in the scanning electron microscope. Bar, 5 µm.

ing was present in an irregularly bent mutant of *B. subtilis*⁵. The thickening was attributed to an altered and irregular growth pattern⁴ or to lack of wall expansion due to insufficient lytic enzyme⁵. Without an explanation for the development of a regular pattern of wall thickening, these suggestions alone cannot account for long regular helix formation.

Helical shape may also occur if the cell wall consisted, at least in part, of fibres wound in a helical manner around the cell. If the quantity or quality of right- and left-hand wound fibres were unequal, and if the fibres were in a state of tension or compression, the cell might assume a helical shape, the direction of which would depend on which class of fibre predominated. This characteristic has been mentioned in a theoretical analysis of the structure of body walls of certain worms⁶.

Helical shape in a bacterium may occur in the following circumstances.

(1) If, in a cell wall normally composed of unequal quantities or qualities of right- and left-hand wound fibres, tension or compression forces along the fibres were introduced through imbalance in wall formation, expansion and turnover.

(1*a*) As (1) but fibres are normally wound in only one direction in the same wall area or at the same time. Helical cell shape may then also result from the inclusion of abnormal contrarily wound fibres.

(2) If, in a cell wall normally containing equal quantities and qualities of right and left hand wound fibres under tension or compression, the equality is disturbed.

(3) If, in a cell wall where the fibres are not normally helically arranged (that is, are parallel or at right angles to the cell's long axis), the pattern is disturbed to give unequal right- and left-hand wound helical components.

None of these models involves anchorage of cell ends and therefore they can explain our observations.

There is evidence that Gram positive cell wall material is synthesised in the form of fibres. During early stages of reversion

of *B. licheniformis* protoplasts to bacilli, murein and teichoic acid are in the form of distinct fibres⁷. In addition, a fibrillar structure has been seen in sections¹ although the latter observation is not a reliable guide to wall structure. Pooley⁸ suggested that wall material in *B. subtilis* is arranged in somewhat discrete fibres or sheets to facilitate the complex expansion and reorganisation that accompanies growth.

The glycan chains of *B. subtilis* peptidoglycan are too long to be oriented radially in the wall⁹ but are short (< 200 nm (ref. 9)) compared with the cell circumference (~1.8 µm, unpublished data) and fibres⁷. It is, however, easily conceivable that fibres consist of several overlapping cross-linked glycan chains. They could be formed in a manner comparable with the apparent mode of cellulose fibre synthesis in higher plants¹⁰, that is, by membrane-embedded enzyme complexes. Whole fibres could be cross-linked after deployment in the wall.

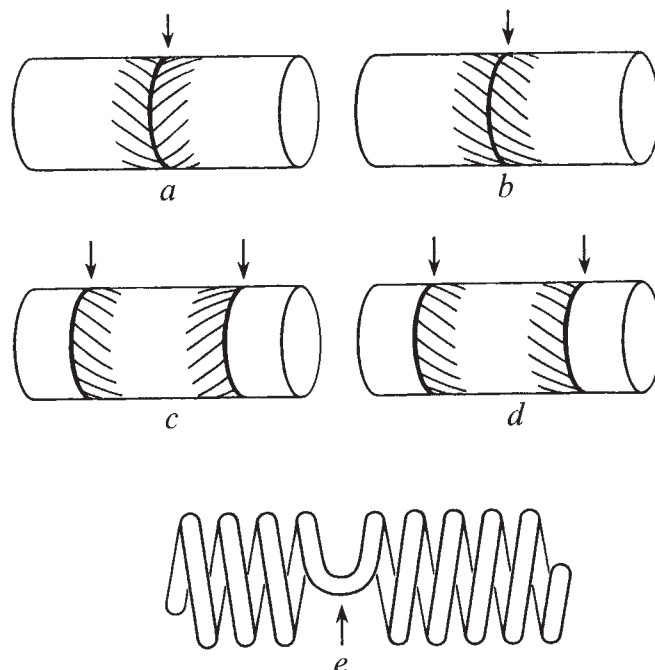
The ease with which wall metabolism can be unbalanced is not surprising considering its biochemical and spatial complexity. The fact that helical cell shapes have been observed by ourselves, Mendelson² and Highton and Hobbs⁴ in a variety of circumstances suggests a helical component in normal as well as mutant wall synthesis. It is not possible at present to decide which, if any, of the above models resembles the true situation but models 2 and 3 seem less likely because equality of right- and left-handed fibres would occur through random assortment. Interference with wall synthesis in such cases is likely to increase randomness and not create a non-random situation. Model 1*a* is consistent with Mendelson's suggestion of helically oriented wall polymers².

If growth sites are real entities in bacillus walls¹¹⁻¹³, it is not difficult to envisage a mechanism to ensure homogeneously oriented wall fibres through head-to-tail alignment, in a ring around the cell, of the enzymes (Fig. 2*a-d*). If growth is diffuse, orientation of new fibres may be guided by pre-existing fibres.

A helically-distorted filament of cells containing a boundary between regions of right- and left-handed fibres should appear as in Fig. 2*e*. Such forms were in fact found (Fig. 1*f*). However, the regular occurrence of such boundaries, as implied in Fig. 2*a* and *c*, does not seem to occur, at least in condition (3) above, since uninterrupted helices several cells long are frequent (Fig. 1*a* and *f*).

The type of model discussed may have a role in propagating

Fig. 2 *a-d*, Some possible patterns of wall fibre formation at growth sites (↓); *a* and *b*, double-sided sites; *c* and *d*, single-sided sites; *e*, expected appearance of helical cell filament at boundary (↑) between regions of oppositely wound fibres.



the normal very regular shape of *B. subtilis* and could readily explain, in principle, the normal helical shape of spirilla¹⁴.

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Neoplastic transformation of hamster embryo cells irradiated *in utero* and assayed *in vitro*

INDUCTION of neoplastic transformation *in vitro* by X rays^{1–5} and neutrons¹ has been reported. Using short term cultures of hamster embryo cells we found previously^{1–4} that transformation by X rays can be detected with doses as low as 1 rad. The rate of transformation increases with dose reaching a peak of 1% between 150 and 300 rad. This frequency of neoplastic transformation *in vitro* is much higher than the frequency of radiation-induced tumours observed after exposing animals to similar doses of radiation⁶. We report here that malignant transformed cells can be obtained from embryos irradiated *in utero* and assayed *in vitro* and that the frequency of transformation is at least tenfold lower than that obtained when the irradiations are performed *in vitro*, and thus closer to the incidence in animals.

Pregnant Syrian hamster strain LSH (Lakeview) were irradiated at 12 d of gestation with 300 rad of X rays. The embryos were surgically removed within 30 min after irradiation. Passaging was carried out as described below and in Table 1. We tried to conserve in culture as many of the original cells as was technically possible. The cultures were passaged twice a week and each flask was handled individually. At every passage half the population of the freshly dissociated cells, resuspended in fresh medium, was replated back into the original flask while the rest of the cells were seeded into a new flask containing 5×10^4 feeder cells (syngeneic cells irradiated with 4,000 rad (ref. 7)). After the third passage cells were replated only into the flasks from which they had been removed. Embryos at 12 d of gestation from non-irradiated hamsters of the same litter served as controls and were cultured as above.

Transformed cells were initially identified by their altered morphology and growth patterns^{1–4}. An additional criterion of transformation is the ability of cells to grow in low serum concentration, which will not support the growth of normal cells. Cells which, from morphological considerations, seemed to be transformed were isolated and grown into mass cultures in medium fortified with either 10% or 1% foetal calf serum. The ability of the cells to proliferate at the lower serum concentration served as an immediate and simple check of their transformed nature.

Within 30–40 d (7–9 passages) after primary cultures had

been established from irradiated embryos, cell transformation was observed in four out of five experiments. In each experiment five to six foci were isolated and a number of cell lines were established. Two established transformed lines designated LSH 1 and LSH 5, each from a different experiment, have been studied extensively. The results of these investigations are summarised for LSH 5 in Table 1.

Chromosome analysis carried out at early passages after transformation (see Table 1) indicated that the modal number of the transformed cells was close to the diploid, 44. The number of chromosomes per cell in LSH 1 ranged from 40 to 56; modal chromosome number 46 (in 40% of the cells), and in LSH 5 the range was 38–60, modal chromosome number 48 (in 30% of the cells). There was no difference between the banding patterns of specific metaphase chromosomes from the transformed cells as compared with the normal.

While the two lines differed from one another in their saturation density, they both reached a higher saturation density than the normal cells in the two sets of conditions. The doubling time of the transformed cells of both cell lines was about 16 h which does not differ greatly from that of normal cells grown in optimal condition (Table 1).

A scanning electron microscopy study indicated that the cells which express transformation *in vitro* following X-ray irradiation *in utero* are similar to those transformed *in vitro*³.

Changes in cellular surface microstructure, following neoplastic transformation, result in the ability of the transformed cells to be agglutinated by low concentrations of plant lectins^{8–10}. The normal counterparts are not agglutinable at similar concentrations (Table 1). The ability of transformed cells to grow in agar is associated with their loss of anchorage dependence. Normal cells derived from solid tissue do not form colonies in semi-solid agar. Following an incubation period of 2 weeks in 0.33% agar¹¹ the control plates showed no sign of colonies, while the plates containing the transformed cells exhibited distinct colonies with a plating efficiency of 5% and 10% at passage nine for LSH 1 and 5, respectively.

We investigated some biochemical markers associated with the neoplastic state of the cells; namely the fibrinolytic activity

Fig. 1 Thin-layer chromatogram of gangliosides from normal hamster embryo cells and X-ray transformed LSH 5 cells. Lane 1 gangliosides in control cells, lane 2 ganglioside standards, and lane 3 gangliosides in X-ray transformed cells. Note that in the transformed cells gangliosides higher than GM3 are absent. Analysis was by Drs P. H. Fishman, R. O. Brady and R. M. Bradley, using published procedures¹⁷.

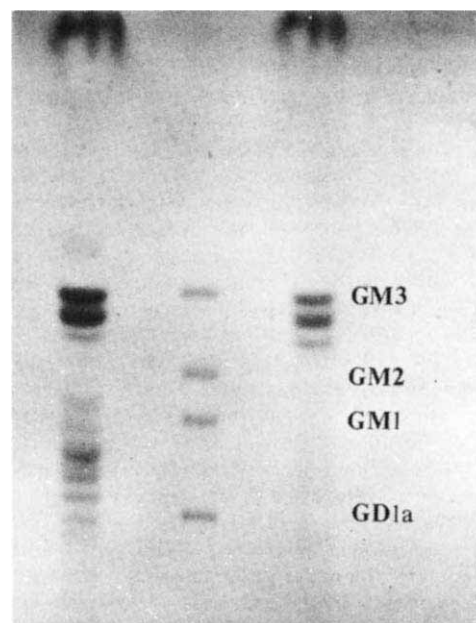


Table 1 Characteristics of LSH 5 cells transformed *in utero* by X-irradiation

	Normal (Secondary culture)	Transformed (4th to 30th passage after isolation)	Tumour (1st to 10th passage) after reculturing
Karyotype	Diploid Monolayers	Near diploid Multilayers	Aneuploid Multilayers
Growth in culture			
Saturation density no. of cells cm ⁻²			
in 10% serum	7.6 × 10 ⁴	1.8 × 10 ⁵	1.5 × 10 ⁵
in 1% serum	Minimum growth	10 ⁵	10 ⁵
Doubling time			
in 10% serum	16 ± 2 h	14 ± 2 h	16 ± 2 h
in 1% serum	90 ± 5 h	24 ± 2 h	25 ± 2 h
Morphology—scanning electron microscope	Flat and regular surface features except during mitosis	Pleomorphic with complex surface features throughout the cell cycle	Pleomorphic with complex surface features throughout the cell cycle
Agglutinability by con A (20 µg ml ⁻¹) and WGA (50 µg ml ⁻¹)	—	+	+
Plating efficiency			
in 10% serum	2.75 ± 1%	52.5 ± 5.5%	49.5 ± 6.5%
in 1% serum	0.05 ± 0.05%	5.5 ± 2.5%	6.0 ± 2.5%
Efficiency in 0.33% agar	0	10%	30%
Plasminogen activator	low	high	high
Lysosomal hydrolases: phosphatase	low	high	high
Production of MIF	absent	high	high
Gangliosides	Presence of full range of gangliosides	Absence of gangliosides higher than GM ₃	Absence of gangliosides higher than GM ₃
Presence of radiation leukemia virus	—	—	—
Tumorigenicity (invasive malignant sarcomas and carcinomas)	none	29/30	Transplantable 12/12

Cells were cultured from a malignant sarcoma which grew in the syngeneic host following subcutaneous (s.c.) injection of 10⁵ of the transformed cells and from normal embryonic cells of a secondary culture. Irradiations (see text) were performed in a parallel opposed set-up, so that animals were irradiated simultaneously from above and below with two X-ray tubes operated at 180 kV, 30 mA, with added filters of 0.28 mm aluminium and 0.28 mm copper. The dose rate in the position occupied by the animals was 250 rad min⁻¹. Embryos were removed within 30 min of irradiation. Primary cultures were established by progressive dissociation of the minced fresh tissue¹¹. Cells (2 × 10⁷) were seeded into eight 75 cm² flasks (Falcon) and incubated at 37 °C in a humidified incubator with a constant flow of 5% CO₂ in air. Medium, composed of Dulbecco's modified Eagle's medium fortified with 10% foetal calf serum (Grand Island Biological), was replaced 12 h after seeding and the cells were allowed to grow to confluency. Cells were passaged twice a week at a ratio of 1:2 (see text). Transformed foci were observed at passages 7–9 in 6 or 10 of 64 flasks. Foci were isolated using reported techniques⁷. Chromosomes were analysed at the 6th passage after isolation using quinacrine fluorescent banding²¹. Saturation densities and doubling time were determined by seeding 10³ cells in 60-mm Petri dishes in medium containing 1% or 10% foetal calf serum and following cell growth daily with a Coulter counter. Methods used for scanning electron microscopy³, testing for concanavalin A (con A) 20–50 µg ml⁻¹ and wheat germ agglutinin (WGA) 20–50 µg ml⁻¹ induced agglutination¹⁰, colony formation in 0.33% agar¹¹, testing plating efficiency (PE; PE = (No. clones counted/no. cells plated) × 100), and assays of fibrinolytic activity (plasminogen activator)¹², migration inhibitory factor (MIF)¹⁵, lysosomal acid hydrolases¹⁶, ganglioside content^{17,18} and presence of radiation leukaemia virus¹⁹ were as reported before. Tumorigenicity was established by s.c. injection of 10³–10⁷ cells in 0.1 ml of serum-free medium into 2-week-old syngeneic hosts or into the cheek pouch of allogeneic LHC strain hamsters. Portions of the tumours were fixed and stained for histopathological examination and other portions were implanted s.c. in 2-week-old syngeneic hosts; a third portion of the tumours was cultured *in vitro*¹¹. Cultured tumour cells were studied as described for the transformed cells. In quantitative studies cells were freshly dissociated from embryos irradiated with 300 rad *in utero* and cloned on feeder cells^{1,2}. Seeding levels were 2 × 10³–10⁴ cells per 60-mm Petri dish (Falcon); 400 dishes were used in each assay. Non-irradiated embryos cultured in the same manner served as controls. The criteria for transformation and the analysis of transformation rate were as before^{1–4}.

of the transformed cells, the release of macrophage inhibitory factor (MIF), the pattern of cellular lysosomal hydrolases and the ganglioside content of the cells. Fibrinolytic activity^{12, 13} of the transformed cells was over 20-fold greater than normal; MIF activity^{14, 15} was confined exclusively to the transformed cell population. Out of 15 cellular acid hydrolases which were determined fluorometrically¹⁶, only acid phosphatase seemed to be significantly elevated in the transformed cells (a twofold increase over the normal). While the full range of gangliosides^{17, 18} was present in the normal cells the transformed cells showed a complete absence of gangliosides larger than GM₃ (see Fig. 1).

Experiments performed by Drs H. S. Kaplan and A. Dèclève, using a reported immunofluorescent assay¹⁹, demonstrated that there was no radiation leukaemia virus (Rad LV) present in the radiation transformed cell lines.

The critical assay for the malignant nature of transformed cells is their ability to give rise to tumours when injected into appropriate hosts. The cells transformed *in utero* by radiation and cultured into cell lines *in vitro* have all given rise to tumours in syngeneic LSH hamsters and also in hamsters of the LHC strain. All animals were 2 weeks old when injected. The tumours were characterised as non-metastasising malignant invasive sarcomas or carcinomas (Fig. 2). They grew progressively and killed the animals.

The tumours are transplantable and have been cultured *in*

vitro. The morphological and biochemical properties of the cells cultured from the tumours are similar to those of the original transformed cells (Table 1).

Malignancy varied from one transformed cell line to another (details to be reported elsewhere). In the 2 lines discussed above 10⁵ cells of LSH 5 and 10⁶ cells of LSH 1 can induce a palpable tumour, 2 cm in diameter, within 2 weeks after injection. Our quantitative studies on transformation *in utero* are outlined in the legend to Table 1. In three experiments we observed a transformation rate ranging from 0.07 to 0.1 following a dose of 300 rad. This frequency is about tenfold lower than that obtained in earlier *in vitro* experiments. It is closer to the frequency observed in some of the studies carried out *in vivo*⁶.

Several intriguing questions are evoked. Does the host mediate in modulating transformation by radiation? Is there a repair of transforming events before they can be expressed? How significant is the state of cells during irradiation in determining the rate of transformation? It is well known from *in vitro* studies that cell replication is required for fixation of the transformation^{20, 21}. With the *in vitro* technique, cells are seeded as single cells with ample opportunity to divide. In addition, of course, they are not in contact with one another, and constitute a mixture of cell types from many tissues. *In utero* the situation is quite different; the embryonic cells are irradiated as tissues where there is cell to cell contact in

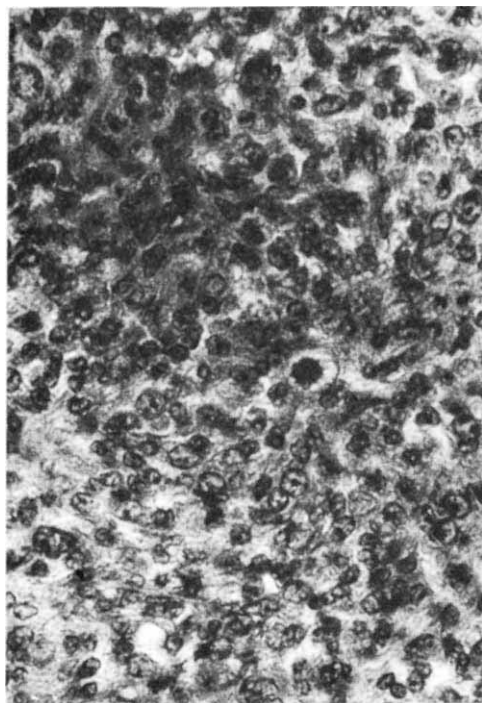


Fig. 2 Section of 3-week-old malignant invasive carcinoma growing in a Syrian hamster following s.c. injection of 5×10^5 cells of a cell line (LSH 5) transformed *in utero* by X-ray irradiation ($\times 220$).

tissue-specific arrangements, and where the rate of cell replication varies with the tissue. It remains to be seen which of these factors, if any, is responsible for the lowered yield of transformed cells characteristic of *in utero* as opposed to *in vitro* irradiation.

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Local inhibition of centripetal particle transport where LETS protein patterns appear on 3T3 cells

WE try here to establish a link between a motility phenomenon in animal cells and the presence of the so-called 'large external transformation-sensitive (LETS)' protein on the surface of the cells. Through studies of this protein a new and still puzzling aspect of animal cell surfaces became apparent. Originally discovered as a major surface glycoprotein of molecular weight 220,000–250,000 which disappeared on cell transformation (for review, see ref. 1), it has since become quite likely that various differently named surface glycoproteins—galactoprotein a (ref. 2), fibroblast surface antigen³, Zeta protein⁴, 'cell-surface protein' (CSP)⁵, and 'cold-insoluble globulin' (CIG)^{6–8}—which were studied independently by different investigators, are identical to or at least very closely related to LETS protein.

If studied by the methods of indirect immunofluorescence, the distributions of LETS protein on the dorsal surface of fibroblastic cells show intriguing aspects. The protein distribution patterns, or at least their antigenic availability, seem to originate from cell-cell contact areas⁹. Later on, the protein can cover large cell surface areas in the form of streaks, indicating a major inhomogeneity in the cell surface composition. Furthermore, it does not remain localised on the cell which produced it. In several-day-old cultures it can form three-dimensional fibrous networks which span between cells throughout the whole culture. The biological function of this protein is not yet entirely clear; however, it has been found to restore normal cell morphology in transformed cells¹⁰, to correlate inversely with tumorigenicity⁹, and to change from a dense fibrillar network in prefused myoblasts (Yaffe's rat myogenic cell line L8) to a sparse dot pattern in myotubes¹¹.

The centripetal surface flow^{12–15}, that is, the radial transport of particles attached to the cell surface along that surface at speeds of $0.2\text{--}3\text{ }\mu\text{m min}^{-1}$ towards the perinuclear region in cultured fibroblastic or epithelial cells (see Fig. 2), is another quite puzzling surface phenomenon. It is not restricted to the cell body but is also expressed on the surface of lamellipodia, blebs and even in the only $0.2\text{-}\mu\text{m}$ thin needle-shaped filopodia^{16,17}. At present, neither a molecular mechanism nor a cellular structure are known which could explain this unidirectional and radial particle movement. Although it is generally believed that the whole cell surface flows constantly towards the nucleus^{15,21}, it is not yet ruled out that the particle movement may be induced only locally at the site of encounter between the cell surface and non-cellular material.

Assuming a constant centripetal flow, one might expect that fibrillar surface components like LETS protein eventually have to orient themselves parallel to the radial flow lines. The complex distribution patterns of LETS protein of all cell lines investigated so far seemed hard to reconcile, however, with a radial centripetal flow (see Fig. 1). One may suspect, therefore, that the flow has ceased in areas covered with LETS protein.

Indeed, the observation of movements of fluorescent antibody-labelled LETS protein patterns in live 3T3 cells showed only slow distortions of the fibre arrangement (Fig. 1). LETS protein fibres could stretch, shorten, fold, move towards or away from the nucleus; however, the combined movements seemed to be unidirectional and very slow. We also observed gold particles of 2,000–4,000 Å diameter (particle clusters up to 1 μm diameter)^{16,17} which had attached to the dorsal cell surfaces inside an area covered with LETS protein, and did not find that they moved centripetally.

One may argue that these experiments do not reflect the natural cell surface movement any more, because the antibody treatment, necessary for the visualisation of LETS protein patterns, influenced the cells. Therefore, we allowed 3T3 cells to first centripetally transport the gold particles, and subsequently

detected the LETS protein on the dorsal cell surface by indirect immunofluorescence with CIG antibody. Figure 2 shows in the case of isolated cells two stages of the radial and centripetal movement of the particles from the cell edge where they were picked up, towards the perinuclear region (Fig. 2a) where they accumulated and eventually became internalised (Fig. 2b). It took 4–6 h before 10–20% of the cells had accumulated sufficient particles to show recognisable particle rings around the nucleus. The rings of particles (Fig. 2a) were not always completely closed, not even in isolated cells.

Four hours after exposure to gold particles—that is, before the particles became internalised, we fixed 3T3 cell cultures on glass coverslips and stained them for LETS protein. The result is shown in Fig. 3. Cells which were able to accumulate the gold particles all round the nucleus in the form of a ring showed only a few patches or no LETS protein at all (Fig. 3a, cell a), whereas cells with extended patterns of LETS protein still had the particles scattered randomly all over their surface (Fig. 3a, cells b and c). We also observed quite a few cells with incomplete gold rings. In many such cases the ring segment marked a cell-surface sector without LETS protein, whereas the complementary sector contained extended LETS protein patterns (Fig. 3b). We never found a big particle cluster on top of an extended LETS

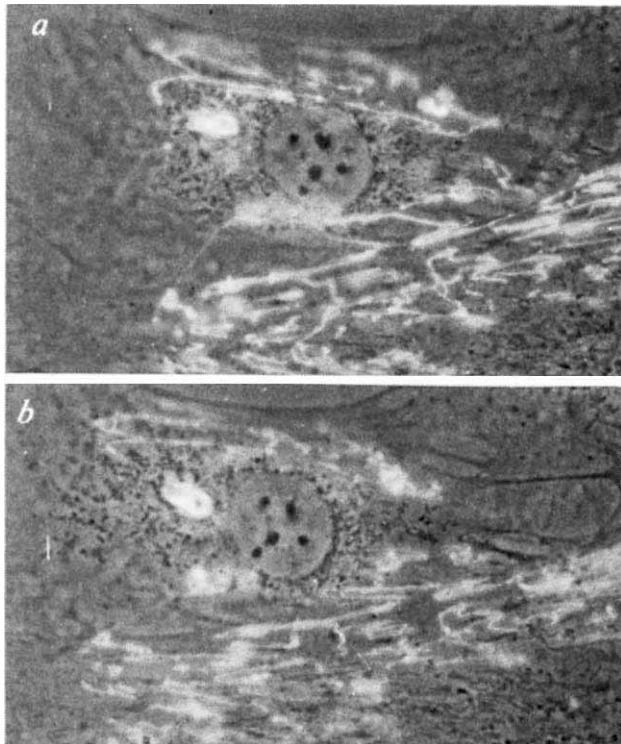


Fig. 1 Movements of LETS protein patterns on live 3T3 cells. Bar indicates 10 μ m. 3T3 cells grown for 3 d on glass coverslips in DME + 10% calf serum were washed in DME, incubated at 37 °C for 15 min in rabbit anti-cold-insoluble globulin antibody (anti-CIG antibody) in DME (dilution 1:80). Anti-CIG antibodies bind to LETS protein¹⁸ and also stain the same cellular structure as anti-LETS antibodies^{6,9,11}. CIG antigen purification and antibody preparation were as described previously^{9,11}. After a further washing in DME, the coverslips were incubated for 15 min at 37 °C in DME containing fluorescein-isothiocyanate-labelled goat anti-rabbit IgG (Meloy, Springfield, Virginia; dilution 1:20), washed again, and returned to DME + 10% calf serum. Live cells were observed in a Zeiss Photomicroscope III (63x; N.A. 1.4) as described previously²². *a*, LETS protein pattern on the dorsal surfaces of two adjacent 3T3 cells. Pictures were taken such that a phase-contrast image was created by the illumination from below the stage while an epilluminator produced and superimposed the fluorescent image. *b*, Same pattern 48 min later.

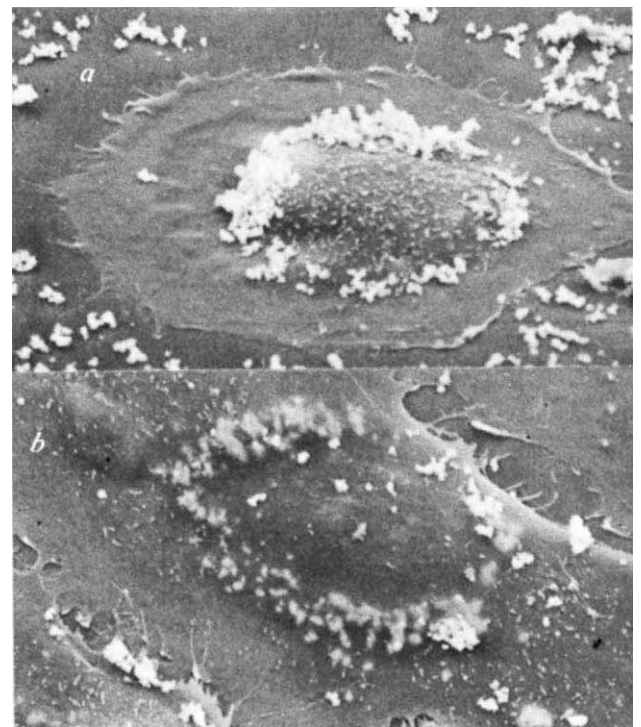


Fig. 2 Centripetal transport of gold particles in 3T3 cells. (Scanning electron microscopy; bar indicates 10 μ m in horizontal direction.) Swiss 3T3 cells (Dr H. Green, MIT) were grown for 2–3 d on glass coverslips in DME supplemented with 10% calf serum. After adjusting a colloidal suspension of gold particles^{16,17} to 290 mOsm by adding 49 mg ml⁻¹ D-glucose (Baker, Phillipsburg, New Jersey), the 3T3 cell cultures were rinsed in PBS and incubated for 5 min in gold suspension at 37 °C. During this time the particles settled on top of the substrate and dorsal cell surfaces. The density of adherent particles is much higher in the substrate than on the cell surfaces. After exposure to gold particles, the coverslips were washed, incubated again in DME + 10% calf serum and returned to the incubator. Cells of cultures which are 3 d old or younger often retracted slightly after the staining procedure, but reverted rapidly to normal morphology. *a*, Isolated 3T3 cell with a complete ring of particles (arrow) 4 h after exposure to the staining procedure (tilt angle 60°). *b*, Another cell 22 h after staining. Between 12 and 22 h the rings of particles become internalised, as seen by the blurred image of the ring which is now below the cell surface (tilt angle 45°).

protein pattern. Clusters were often located, however, at the edge of a pattern.

These observations suggest that the particles cannot move radially into or inside areas where LETS protein covers the cell surface in the form of extended patterns. One would expect, therefore, that removal of LETS protein from the cell surface would increase the centripetal transport of particles.

During a 10-min treatment of a 3T3 cell culture with 5 μ g ml⁻¹ trypsin in Dulbecco's Modification of Eagle's medium (DME), the predominant component removed from the dorsal cell surfaces is LETS protein^{4,19,20}; yet the cells show no detectable shape alteration in response to this treatment. Therefore, 3T3 cells, grown for 5 d on glass coverslips, were washed in DME and incubated at 37 °C for 10 min in DME containing 5 μ g ml⁻¹ trypsin (Miles Laboratories, Kankakee, Illinois) and then returned to their previous culture medium for 45 min. Control cultures were similarly treated with DME or DME containing 5 μ g ml⁻¹ thrombin (Dr J. Fenton, Albany, New York) since it has been shown earlier that neither treatment affects the LETS protein²⁰. Subsequently, the cells were exposed to gold particles as described in Fig. 2, and returned to their previous culture media for 4 h. After fixation of the preparations, we determined the percentage of cells which had accumulated particle clusters. In 20 randomly selected fields we counted

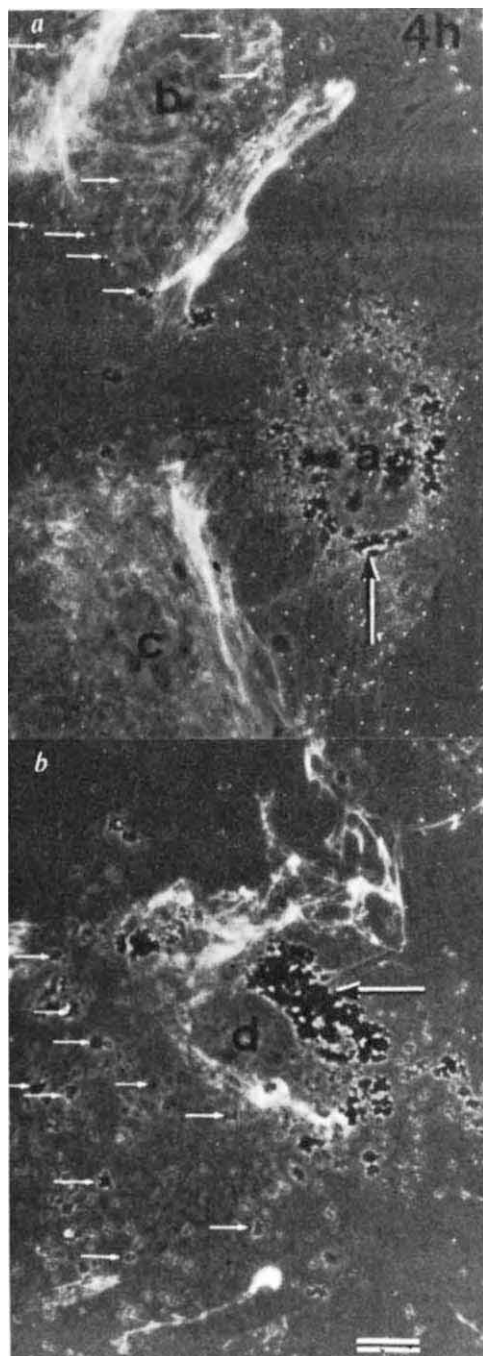


Fig. 3 Mutual exclusion of extended LETS protein patterns and areas with centripetal particle transport (bar indicates 10 μm). 3T3 cells were grown for 5 d without medium change on $12 \times 12\text{-mm}$ glass coverslips and incubated in gold particle suspension as described in Fig. 2. Four hours later, the cells were fixed in 3.5% formaldehyde in PBS and incubated for 30 min at 37°C with anti-CIG antibody in PBS (dilution 1:80). After a further washing in PBS, the coverslips were incubated for 30 min in DME containing fluorescein isothiocyanate-labelled goat anti-rabbit IgG (dilution 1:20), washed again, mounted and observed in the same conditions as described in Fig. 1. *a*, 3T3 cell (a) with a complete ring of gold particles (arrow) and no LETS protein on the surface, and two other cells (b, c) with extended LETS protein nets but no particle ring. Randomly scattered small particle clusters are indicated by small white arrows. Big particle clusters appear black. *b*, Incomplete particle ring (arrow) with LETS protein patterns covering the complementary ring segment. Opposite to the ring a 'barrier' of LETS protein can be seen and behind it many particle clusters (arrow points to one of them), which were not moved towards the nucleus (d).

approximately 300 cells in each preparation and found the following percentages of cells with particle clusters. (Standard deviations describe variations over the selected fields). DME + $5 \mu\text{g ml}^{-1}$ trypsin: 64% (s.d. 15%); DME + $5 \mu\text{g ml}^{-1}$ thrombin: 20% (s.d. 8%); DME: 13% (s.d. 10%). The three- to four-fold increase in particle accumulation by the trypsin-treated cells compared with controls suggests that removal of LETS protein from the cell surfaces does increase the speed and extent of the centripetal particle transport around the cells' perimeters.

It seems, therefore, that the centripetal transport of surface-attached particles and, perhaps, the surface flow itself are locally inhibited by those surface networks which are predominantly built of LETS protein. It remains to be seen whether there is an inhibitory effect of LETS protein on cell motility in general, as was originally postulated by Hynes²³. In view of the observation that LETS protein networks seem to originate from cell-cell contacts, the possibility that those networks inhibit surface flow seems to offer an intriguing mechanism of cell-cell interaction: It would suggest a way by which the surface movements in two or more cells become altered depending on direction and extent of their mutual contacts.

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Complement enhances antiviral antibody-dependent cell cytotoxicity

ANTIBODY-DEPENDENT cell cytotoxicity (ADCC) is an *in vitro* phenomenon that can be demonstrated with a variety of target cell systems and can be mediated by several types of effector cells^{1–3}. The effectors in ADCC must bear a surface receptor for Fc, and this receptor binds with antibody which is in turn bound to the target cell. Only certain classes and subclasses of immunoglobulins can sensitise cells for ADCC^{4,5}. Although the presence of a Fc receptor is necessary for ADCC, it is not sufficient since cells bearing such receptors may fail to mediate ADCC against a

particular target^{1-3,6,7}. Similarly, union of effectors with targets by means of a different receptor such as the complement receptor may not result in cytotoxicity^{8,9}. Whether the efficiency of different cells at mediating ADCC depends on the number, type or affinity of Fc receptors, whether a cooperation between different types of surface receptors is needed¹⁰ or whether some property of the target cells decides the outcome of contact between effectors and targets, has not been established. It is likely that

ADCC occurs only if a sufficient union is established between effector and target cell. Such a union could be generated by bonds between the target cell and two types of surface receptors on the effectors—such as the Fc receptor and the complement receptor, both of which are present on effector cells¹⁰⁻¹². Using herpes-virus infected target cells we here provide support for this hypothesis and show that the addition of complement, at levels below those required for antibody complement lysis, considerably enhances

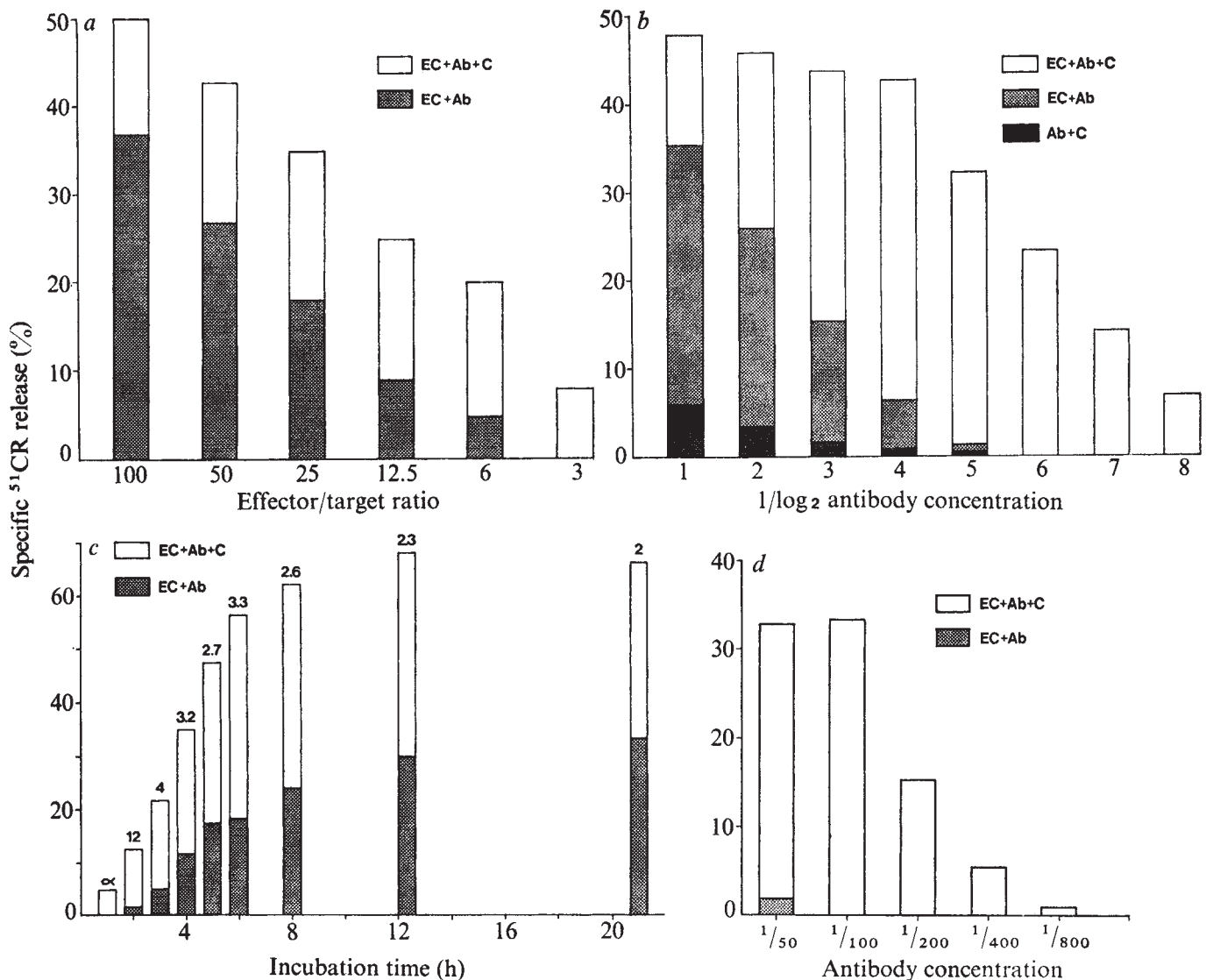


Fig. 1a, Enhancement of neutrophil-mediated ADCC at various effector-to-target cell ratios by rabbit complement. Neutrophils were collected from the bovine mammary gland and purified¹⁴. Target cells were infected with IBR virus and labelled with $\text{Na}_2^{51}\text{CrO}_4$ 16 h before the start of assays⁶. Target cells were sensitised with a 1/800 final concentration of hyperimmune bovine anti-IBR serum. Rabbit complement was added at a final concentration of 1/70. At this concentration, lysis of antibody-sensitised target cells in the absence of effector cells was 2% above background (12% in a 6-h assay). Assays were performed in microtitre trays⁵. The percentage specific ^{51}Cr release was computed by the formula

$$\text{specific release (\%)} = \frac{\text{radioactivity in test supernate} - \text{control}}{\text{total releasable radioactivity} - \text{control}} \times 100.$$

The neutrophils in the absence of anti-IBR serum failed to give release of ^{51}Cr above background either in the presence of media alone or media plus 1/70 rabbit complement. EC, effector cells; Ab, anti-IBR serum; C, complement. **b**, Enhancement by complement of neutrophil-mediated ADCC at different concentrations of anti-IBR serum. Assay as for (a). The assay was run at a neutrophil/target cell ratio of 100:1. The final antiserum concentration at level 1 was 1/100 and 8, 1/12,800. Rabbit complement was included at a final concentration of 1/70. **c**, Enhancement by complement of neutrophil-mediated ADCC at different durations of assay. The neutrophil/target cell ratio was 100:1, the final concentration of sensitising anti-IBR serum 1/800 and rabbit complement 1/70. Figures at the top of each column refer to the magnitude of the specific release in the presence of complement compared to that without complement. The background releases ranged from 6.7% at 1 h to 18.4% at 21 h in media alone and from 8.9% at 1 h to 22% at 21 h in the presence of complement. **d**, Enhancement of lymphocyte-mediated ADCC by rabbit complement. Lymphocytes were collected from peripheral blood by Ficoll-Hypaque flotation and were further purified by passage over glass wool to remove adherent cells¹³. The preparation gave a low level of specific ^{51}Cr release (2%) at the highest concentration of serum tested, possibly due to some remaining macrophages or neutrophils. The assay was performed for 6 h in the presence or absence of 1/70 final concentration of rabbit complement. The level of specific release by complement in the absence of effector cells was 5% at 1/50, 3% at 1/100 and 2% at 1/200 of anti-IBR serum, respectively.

ADCC and, furthermore, permits cells to mediate ADCC that in the absence of complement could not do so.

Previously, we have described the cell types and conditions necessary to demonstrate ADCC against bovine antibody sensitised bovine target cells infected with infectious bovine rhinotracheitis (IBR) virus—a bovine herpesvirus⁶. We have compared several cell types and have shown that neutrophils perform best and that lymphocytes fail to mediate ADCC^{1,3}. Lymphocytes, however, do show activity against erythrocyte target cells⁶. We hypothesised that since the cells that mediate ADCC had complement receptors as well as Fc receptors^{11,12}, then if bonds between effectors and targets were additionally engaged by the complement receptor then cytotoxicity should be enhanced. Such enhancement should be more apparent in those conditions where cytotoxicity was submaximal, such as low effector-to-target cell ratios, low concentrations of sensitising antiserum and short term assays. That the addition of complement does enhance ADCC is shown in Figs 1–3. Also shown are data that support the predictions enhancement was more apparent at low effector-to-target cell ratios (Fig. 1a), low concentrations of sensitising antiserum (Fig. 1b) and short duration assays (Fig. 1c). One explanation for the enhanced cytotoxicity could be that in addition to ADCC we were also observing antibody-complement mediated cytotoxicity. This possibility was considered remote since the levels of complement used in the experiments either failed to exhibit or gave only low levels of cytotoxicity against targets in the absence of effector cells. At high complement concentrations, however, such antibody-complement cytotoxicity was observed, indicating that the material did possess complement activity (data not shown). Furthermore, the rabbit complement preparation was not providing an additional sensitising antibody since heat inactivated material failed to give enhancement.

Not only could the addition of complement lead to enhancement of cytotoxicity, but cell types incapable of mediating ADCC against the virus-infected cells could be made to do so by the addition of complement. Thus lymphocytes alone could not mediate ADCC (Fig. 1d), but ADCC became readily apparent on addition of complement.

These experiments support the hypothesis that multiple binding of effectors to target cells will be followed by enhanced cytotoxicity. It needs to be established if the effect is the result of a more tenacious bond or has some other explanation such as the generation of a biochemical signal that results in target cell lysis. Finally, since ADCC has been considered as an *in vitro* model for an antiviral mechanism of defense⁶, the implications of our results should be considered in terms of the possible part that complement might have in enhancing recovery from certain viral infections.

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Selective incorporation of *H-2* antigenic determinants into Friend virus particles

IN susceptible mice, Friend murine leukaemia virus (FV) produces fatal erythroleukaemia¹ characterised by rapid onset of splenomegaly and viraemia in 4–5 d and leading to the death of the animal in as little as 3–4 weeks. In the replication of this enveloped, C-type RNA virus, assembly occurs at the surface of infected cells where the particles mature by budding². We have examined the question of whether host cell surface glycoproteins are incorporated into the virion as the host cell membrane becomes part of the completed particle during the budding process. In particular, serologically identifiable alloantigens governed by the *H-2K* and *H-2D* genes of the major histocompatibility complex and found on cell surfaces have been sought in lysates of serum-derived FV.

Serum collected from mice infected 10–14 d previously with a high dose of NB-tropic FV was the source of virus. After clarification of the serum at 6,800g, virus was pelleted by centrifugation at 100,000g. The pellet was resuspended in buffered saline containing detergent (Nonidet P-40, 0.25%) to disrupt the virus. This preparation was then examined for the presence of *H-2K* and *H-2D* antigens of the host mouse by determining its capacity to inhibit the cytotoxic activity of various anti-*H-2* antisera. Figure 1 shows the results of an experiment with virus collected from the serum of FV-infected (BALB/c × BALB.B)_{F1} mice (*H-2^b/H-2^d*). The preparation was tested for its capacity to inhibit the complement-mediated lysis of uninfected (BALB/c × BALB.B)_{F1} lymph node target cells with antisera monospecific for either *H-2K^b*, *H-2D^b*, *H-2K^d* or *H-2D^d* antigenic specificities (Table 1). Only lysis by anti-*H-2D^b* antiserum {(A × HTI)_{F1} anti-EL4; *H-2^b/*

Table 1 Anti-*H-2* antisera characteristics

	Alleles recognised	Private specificity
(A × BALB.B) _{F1} anti-BALB/c	<i>H-2K^d</i> , <i>H-2I^d</i>	H-2K.31
BALB.G anti-BALB/c	<i>H-2D^d</i>	H-2D.4
(A × BALB.G) _{F1} anti-BALB.B	<i>H-2K^b</i> , <i>H-2I^b</i> , <i>H-2S^b</i>	H-2K.33
(A × HTI) _{F1} anti-EL4	<i>H-2D^b</i>	H-2D.2
BALB/c anti-BALB.G	<i>H-2D^b</i>	H-2D.2

H-2ⁱ anti-*H-2^b* } was detectably inhibited (Fig. 1a). An identical pattern of inhibition was seen using a second anti-*H-2D^b* antiserum (BALB/c anti-BALB.G; *H-2^d* anti-*H-2^b*) raised against normal spleen cells rather than EL4 tumour cells. Similarly, in virus grown in (C57BL/6 × DBA/2)_{F1} mice, which are also *H-2^b/H-2^d* heterozygotes but with a different genetic background, *H-2D^b* alloantigen but not *H-2K^b*, *H-2K^d* or *H-2D^d* was detected. Neither sham virus preparations made from non-viraemic BALB.B mouse serum nor preparations made from viraemic BALB/c mouse serum produced inhibition of lysis by any of the four antisera (data not shown).

Several criticisms of the experimental techniques used are in order. The augmentation of lysis in the presence of virus above controls is difficult to rationalise (Fig. 1b, c and d). It is not due to the NP-40 detergent, since controls contain identical amounts of detergent. A contaminant in the 100,000g pellet may be responsible, because no such augmentation of lysis was observed in recent preliminary experiments using sucrose density-gradient purified virus. Contamination was not the cause of inhibition of anti-*H-2D^b*-mediated cytolysis, however, for three reasons. First, inhibition of lysis by contaminating plasma membranes would be expected to produce inhibition of all four antisera. Second, BALB.B (*H-2^b*) non-viraemic serum preparations were not inhibitory. And third, BALB/c (*H-2^d*) virus preparations were also not inhibitory. Thus, *H-2D^b* was the

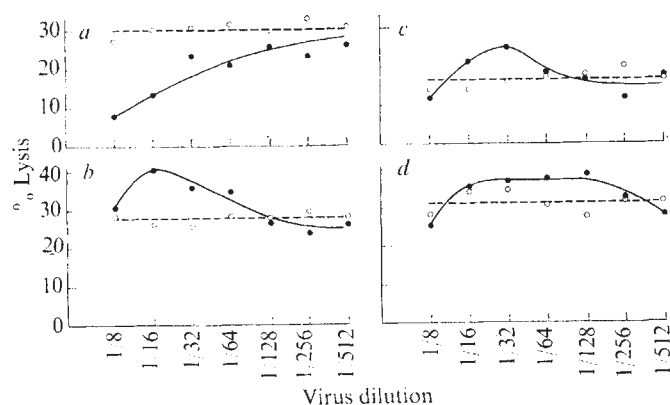


Fig. 1 Serum, prepared from blood collected from (BALB.B $\times$ BALB/c) F_1 mice given 10^4 spleen focus forming units of Friend virus 10–14 d previously, was centrifuged at 6,800g for 15 min and the supernatant recovered. After re-centrifugation of the serum at 100,000g for 90 min, the supernatant was discarded and the pellet resuspended in 0.1 ml phosphate-buffered saline (PBS) containing 1mM EDTA to which was added 0.01 ml of a 2.75% (v/v) solution of NP-40. After 1 h incubation at 4°C, 0.11 ml of a 25% (w/v) bovine serum albumin (BSA) solution was added to neutralise the NP-40 and the entire mixture diluted 1:8 with medium 199 containing 20% foetal calf serum. Control preparations, lacking viraemic serum pellets, contained only PBS, EDTA, NP-40, BSA and 199 with 20% FCS in proportions identical to virus preparations. One-tenth ml of either virus or control preparations was aliquoted into 5-ml Falcon plastic culture tubes in serial twofold dilutions. To these was added 0.1 ml of monospecific anti- $H-2$ antiserum appropriately diluted in 199 with 20% FCS. After 10 min incubation at room temperature, 0.05 ml of ^{51}Cr -labelled (BALB.B $\times$ BALB/c) F_1 mesenteric lymph node cells (10^6 ml^{-1}) was added followed by the addition of 0.05 ml of neat guinea pig serum as a complement source. After 1 h at 37°C in a 5% CO_2 atmosphere, 0.3 ml of PBS containing 10 mM EDTA was added to terminate the reaction, the cultures spun at 300g for 5 min and 0.3 ml of supernatant recovered for counting γ radiation. Curves were generated in the presence of either virus preparations (●) or control preparations (○). Anti- $H-2$ antisera were raised by the alloimmunisations—*a*, (A $\times$ HTI) F_1 anti-EL4, $H-2^a/H-2^b$ anti- $H-2^b$ —anti- $H-2^b$ antiserum; *b*, (A $\times$ BALB.G) F_1 anti-BALB.B, $H-2^a/H-2^b$ anti- $H-2^b$ —anti- $H-2^b$; *c*, BALB.G anti-BALB/c, $H-2^a/H-2^b$ anti- $H-2^b$ —anti- $H-2^b$; and *d*, (A $\times$ BALB.B) F_1 anti-BALB/c, $H-2^a/H-2^b$ anti- $H-2^b$ —anti- $H-2^b$.

sole antigen detected, and it was only detected in virus-containing preparations from mice with the $H-2D^b$ allele.

$H-2$ antigen activity of host cell specificity has been detected before in an enveloped RNA virus. Hecht and Summers³ observed inhibition of cytolysis mediated by anti- $H-2^k$ antisera in the presence of highly purified vesicular stomatitis virus grown in L cells ($H-2^k$). Because of the purification techniques used, they concluded that the $H-2$ antigens were an integral part of the virion. Their finding that both $H-2K^k$ and $H-2D^k$ antigens were present in the virion could be explained by the random distribution of $H-2$ molecules on plasma membrane destined to become virus envelope. Our finding of only one of four host cell $H-2$ specificities in FV suggests that the inclusion of $H-2$ antigens in virus particles is selective rather than random.

The $H-2^b$ haplotype confers relative resistance to FV disease⁴. One gene responsible for this resistance, *Rfv-1*, maps in or near the $H-2D^b$ region⁵. Resistance in BALB.B mice results in strong rejection of implanted cells of syngeneic FV-induced tumour lines, whereas BALB/c mice resist implants of syngeneic FV tumour cells poorly⁶. Characteristic of resistance in BALB.B but not BALB/c mice is the appearance of cytotoxic T lymphocytes specific for the syngeneic tumour. The lytic activity of these BALB.B T cells *in vitro* is subject to $H-2$ restriction; that is, lysis can only be demonstrated against target cells expressing both FV and $H-2^b$ antigens⁷.

Present evidence for the basis of $H-2$ restriction in this and other T-cell killing systems supports the hypothesis that $H-2$

molecules associate with virus molecules on the target cell surface in such a way as to serve as an 'altered-self' marker recognisable by the immune T lymphocyte⁸. Evidence in favour of a physical association at the cell surface between virus and $H-2$ molecules has been obtained in this laboratory with the demonstration that capping of FV antigens on $H-2^b$ tumour cell surfaces causes partial co-capping of $H-2D$ but not $H-2K$ antigens⁹.

If, as the evidence indicates, $H-2$ antigens physically associate with virus antigens on the cell surface, then the appearance of $H-2$ in the virion is most likely the consequence of such an association at the site of virus budding. The inclusion of only one of four possible $H-2$ antigens in the virus, however, implies that not all $H-2$ molecules are equal in their capacity to bind to cell surface virus antigens. The selective inclusion of $H-2$ molecules in C-type virus particles is in keeping with the sporadic detection during biochemical analysis of MuLV of a glycoprotein¹⁰ of 45,000 molecular weight. The molecular weight and irregular detection of this constituent suggests that it may consist in part of host cell $H-2$, inclusion of which in virions depends on its capacity to bind to virus molecules.

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Prevention of experimental allergic encephalitis in Lewis rats by rat and bovine spinal cord proteins

EXPERIMENTAL allergic encephalitis (EAE) is an inflammatory autoimmune disease of the central nervous system (CNS) that is mediated by T lymphocytes sensitised to the organ-specific basic protein (BP) of CNS myelin¹. Lesions of delayed hypersensitivity develop in the brain and spinal cord when sensitised lymphocytes traverse the walls of CNS blood vessels and react with the target antigen in myelin². EAE can be prevented by injecting BP in Freund's incomplete adjuvant (FIA) before challenge, suppressed by injecting BP soon after challenge and treated by injections of BP after clinical symptoms appear³. Prevention, suppression and treatment are immunologically specific for BP and can be dissociated from the production of humoral antibody against BP⁴. The demonstration that pretreatment of guinea pigs with bovine spinal cord protein (BSCP)^{5,6,7} prevented the development of EAE^{8,9} is thus of considerable importance because BSCP is chemically and antigenically distinct from bovine BP and is not encephalitogenic. As pretreatment with SCP induced a state of nonspecific unresponsiveness to bovine BP in the guinea pig, we investigated whether SCP had anti-encephalitogenic activity in the Lewis rat, a highly inbred rat strain used extensively for the study of EAE. We report here that Lewis rats pre-

Table 1 Encephalitogenicity of rat BP, RSCP and BSCP in Lewis rats

Antigen used for challenge* (in FCA)	Amount of antigen injected (µg)	No. sick No. tested	Onset (day)	EAE	Clinical severity†	Histologic severity ‡
Rat BP	20	6/6	14.5		1.8	1.5
Rat BP	30	7/7	12.5		3.4	1.7
Rat BP	50	10/10	13		3.0	1.8
RSCP	300	0/6	—		0	0
BSCP	400	0/8	—		0	0

*Animals were injected in the left hind foot pad with 0.1 ml of an inoculum prepared by emulsifying the appropriate amount of antigen in 0.05 ml of saline with 0.05 ml of FIA containing 200 µg of *M. butyricum*.

†Average scores for clinical symptoms were graded as follows; +, transient weight loss; ++, limp tail; +++, hind end weakness and/or incontinence; + + + +, complete loss of tone in tail (string tail) and paralysis.

‡Average score for lesions in brain and spinal cord graded from 0 to 2+ according to Levine *et al.*¹².

treated with rat SCP (ref. 10) were protected against EAE, as they did not develop the disease when they were subsequently immunised with rat myelin BP in complete Freund's adjuvant (CFA).

Homologous RSCP was investigated first because immunodiffusion studies with anti-BSCP serum and anti-RSCP serum indicated that bovine, monkey, human and rabbit SCP were antigenically closely related^{7,10}, while RSCP was significantly different. The homologous rat BP was used for the induction of EAE. RSCP was purified from rat spinal cord¹⁰. Rat BP containing both the L and the S BP was prepared from rat brain by the method of Deibler *et al.*¹¹.

emulsion containing 30 µg of rat BP and 200 µg of *Mycobacterium butyricum* into pads in the left hind foot.

Table 1 shows that 20 µg of rat BP was sufficient to produce clinical and histological EAE in all animals tested. Disease onset usually occurred 15 d after challenge. Higher doses of rat BP induced more severe disease with onset 12–13 d after challenge. The 30 µg of rat BP used to induce EAE in all pretreated animals was selected as a moderately severe challenge dose. Neither RSCP nor BSCP were encephalitogenic in Lewis rats at levels of 300 and 400 µg, respectively.

Results of pretreatment schedules in which the total amount of RSCP used varied from 300 to 900 µg, and the

Table 2 Prevention of EAE* in Lewis rats by pretreatment with RSCP—effect of variations in dosage and pretreatment schedule

Pretreatment (in FIA)			EAE			
Schedule	Days†	Total dose (µg)	No. sick No. tested	Onset (day)	Clinical severity‡	Histologic severity§
1 × 100 µg RSCP weekly	21	300	1/6	14	0.3	0.5
2 × 100 µg RSCP weekly	21	600	0/6	—	0	0.2
2 × 100 µg BGG weekly	21	600	6/6	12.5	3.1	2.0
3 × 100 µg RSCP weekly	21	900	1/6	15	0.3	0.2
3 × 100 µg BGG weekly	21	900	5/6	12	2.1	0.7
2 × 200 µg RSCP weekly	14	800	0/6	—	0	0
2 × 200 µg BGG weekly	14	800	4/4	13.5	2.7	2.0
3 × 100 µg RSCP	10	300	5/6	13.0	2.8	2.0
3 × 100 µg BGG	10	300	6/6	11.5	3.0	2.0
3 × 100 µg RSCP	5	300	6/6	12.0	3.0	2.0
None			10/10	11.5	2.9	1.9

*Animals were challenged with 0.1 ml of inoculum containing 30 µg of rat BP and 200 µg of *M. butyricum*.

†Number of days between first pretreatment injection and challenge.

‡Average score for clinical symptoms graded 0 to 4+.

§Average score for lesions in brain and spinal cord graded from 0 to 2+.

Male Lewis rats weighing about 250 g were injected in the right hind foot pad at different intervals with 0.1 ml of inoculum prepared by emulsifying the required amount of RSCP with an equal volume of FIA. Control rats were not treated or were injected with bovine gamma globulin (BGG) emulsified with FIA. At the appropriate time, the animals were challenged by injecting 0.1 ml of an FIA

pretreatment interval extended from 5 to 21 d are recorded in Table 2. Pretreatment with three weekly doses of 100 µg of RSCP protected five out of six animals from clinical and histological EAE. Doubling or tripling the amount of RSCP injected over 2 or 3 week intervals gave complete protection of all animals, while control animals that received similar amounts of BGG were not protected.

Table 3 Prevention of EAE* in Lewis rats by pretreatment with different amounts of bovine SCP for 21 d

(Pretreatment (in FIA))		Total dose (µg)	No. sick/ no. tested	Onset (day)	EAE	
Schedule					Clinical severity†	Histologic severity‡
2 × 83 µg BSCP weekly		500	5/6	12	3.0	1.8
2 × 170 µg BSCP weekly		1,000	5/6	14	2.0	1.0
2 × 340 µg BSCP weekly		2,000	1/6	—	0.5	0.2
2 × 170 µg BGG weekly		1,000	5/5	11	3.6	2.0
None			6/6	10.5	3.5	2.0

*Animals were challenged 3 d after the last pretreatment injections with 0.1 ml of inoculum containing 30 µg rat BP and 200 µg *M. butyricum* emulsified in FIA.

†Average score for clinical symptoms graded 0 to 4+.

‡Average score for lesions in brain and spinal cord graded from 0 to 2+.

When the pretreatment intervals were shortened to 10 or 5 d, however, three 100- μ g doses of RSCP did not protect. The results suggest that the amount of RSCP needed for protection is inversely related to the length of the pretreatment period.

Heterologous BSCP also had the capacity to protect Lewis rats against EAE induced with rat BP, although approximately seven times as much BSCP was required to achieve the same degree of protection provided by 300 μ g of RSCP (Table 3). The fact that RSCP and BSCP prevent EAE in the rat as well as in the guinea pig suggests that SCP may be used as a nonspecific anti-encephalitogenic agent.

One possibility that can probably be ruled out is that of specific immunosuppression due to contamination of RSCP with rat BP because SCP was extracted from bovine or rat spinal cord (not de-fatted) with 0.05 M acetate buffers, a procedure that does not solubilise detectable amounts of BP. Furthermore, the amount of rat BP in 1.0 mg of purified RSCP was found not to exceed 30 ng by a competitive inhibition radioassay (unpublished results). This method measures the extent to which binding between a known amount of 125 I-rat BP and a fixed volume of a standard rabbit anti-rat BP serum is inhibited by varying amounts of non-radiolabelled BP using polyethylene glycol to precipitate antibody-bound 125 I-BP^{13,15}. A similar radioimmunoassay kindly performed by Dr V. Lennon of the Salk Institute, showed that 1.0 mg of BSCP contained 15 ng of BP. Thus the 2.0 mg of BSCP required for protection against EAE could not have contained more than 30 ng of BP, an amount far less than the 10,000 ng of BP reported to be the lowest amount capable of preventing EAE in the guinea pig¹⁶.

The mechanism of the protective action of SCP is not clear. Data on the effect of SCP-pretreatment on the lymphocytes mediating EAE suggest that the mechanism is likely to be complex. For example, SCP-pretreated guinea pigs regularly displayed delayed skin hypersensitivity to BP after challenge but there was no correlation between the degrees of cutaneous reactivity and protection from disease⁹.

The protective activity may be related to the location of SCP in nervous tissue. Indirect immunofluorescence studies using rabbit anti-sera showed that SCP is localised in the axons of neurones in the central and peripheral nervous systems and seems to be absent from the surrounding myelin sheaths (unpublished results). Protective activity might also involve non-specific suppression of selected T-cell function by anti-SCP antibody in a manner analogous to that of the anti- θ thymic antibody because RSCP is a component of rat thymus¹⁰.

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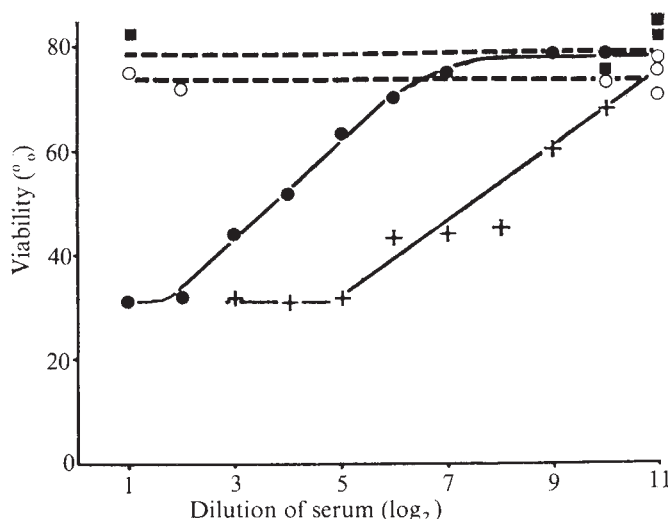
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Gangliosides as surface antigens on cells isolated from the rat cerebellar cortex

GANGLIOSIDES are a class of complex glycolipids characterised by their content of *N*-acetyl neuraminic acid. They are present in many tissues, but are found in highest concentration in those of the nervous system. At one time they were thought to be characteristic of the neurone, but the analysis of cell fractions from a number of species indicates that they are present in glia and myelin as well as in the neuronal elements^{1,2}. The investigation of subcellular fractions suggests that most of the ganglioside is located in the plasma membrane and their functional significance is still uncertain. We have investigated the effect of anti-ganglioside serum on suspensions of cerebellar cells from immature rats. Trypan blue exclusion and 51 Cr release methods were used as indicators of cytotoxic activity. The results indicate that ganglioside is located at the outer surface of the plasma membrane in 60–80% of the cells. The remainder do not seem to exhibit the reactive hapten upon their surface. The experiments also demonstrate the value of complement mediated cytotoxicity as a method of studying surface lipid haptens in the cells of the nervous system.

Suspensions of cerebellar cells were freshly prepared from rats 5–12 d post-natally³. The final suspension contained 5–15 $\times 10^6$ cells per ml in Krebs bicarbonate ring containing glucose, 15 mM, and bovine serum albumin, 0.3% w/v. Antisera were raised in rabbits against the total ganglioside fraction isolated from postmortem human brain tissue. The total ganglioside fraction was isolated from the chloroform-methanol extract by partitioning against saline, and further purified from the upper phase by dialysis and barium precipitation by the method of Wolfe⁴. Rabbits were

Fig. 1 Cytotoxicity of rabbit anti-ganglioside serum for dissociated rat cerebellar cells. ●, Anti-ganglioside serum/cerebellar cells from 6-d-old rats; +, Anti-ganglioside serum/cerebellar cells from 12-d-old rats; ■, control: normal rabbit serum/cerebellar cells from 6-d-old rats; ○, control: normal rabbit serum/cerebellar cells from 12-d-old rats. Guinea pig complement (25 μ l) was added followed by a further 0.5-h incubation. Cytotoxicity assays using Trypan blue (0.2%) were carried out in microtitre trays. The cell suspension was diluted to contain 10^6 cells ml⁻¹. Antiserum (25 μ l) was added to 50 μ l cell suspension and incubated at 37 °C for 30 min. Fresh neat guinea pig serum (25 μ l) containing complement was then added and after shaking, incubated for a further 30 min. For counting, the supernatant was removed and one drop of Trypan blue (0.2%) in Krebs ring added.



immunised with the total ganglioside mixture and either bovine or human serum albumin as carrier, emulsified in complete Freund's adjuvant⁵.

The viability of the cell preparation was generally better than 90% (mode of 96%, range 80–99%). Exposure to normal rabbit serum and guinea pig complement did not affect the viability over a period of hours. Anti-ganglioside serum was found to be cytotoxic for cerebellar cells in the presence of guinea pig complement (Fig. 1). A fraction of the cells, however, were found to be resistant, and even repeated exposure to antiserum and complement failed to increase the proportion of the cells killed (Fig. 2). From Fig. 1 it can be seen that antiserum was more effective against cells from 10-d-old animals than those from 5-d-old animals, although the proportion of cells killed remains approximately constant. The total ganglioside concentration of the rat cerebellum increases sharply 5 d after birth, indicating an increase in membrane concentration⁶. The present findings suggest that this increase may initially be restricted to certain cells, at least for the gangliosides GM1 and GD1b, the suspected antigenic members of the ganglioside mixture^{7,8}.

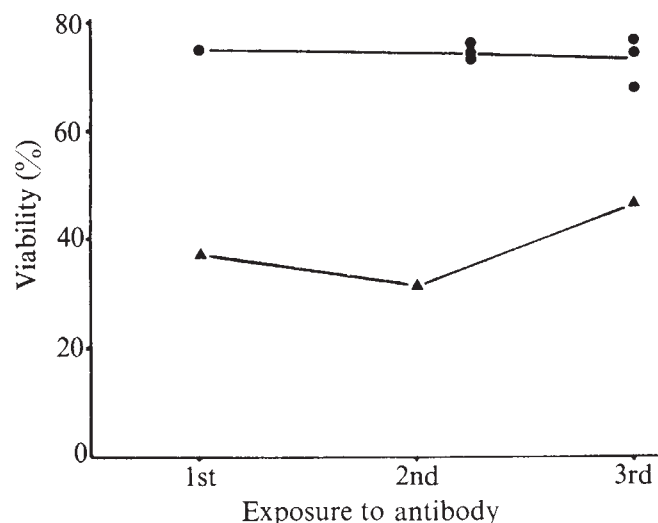


Fig. 2 Effect of repeated exposure of cerebellar cells to anti-ganglioside serum and complement. Viability declines to 30–40% on addition of antibody and reaches a plateau. The surviving cells are unaffected by subsequent treatment with antibody. ▲, Anti-ganglioside serum; ●, normal rabbit serum (control).

The specificity of the antibody for ganglioside was demonstrated by absorption with a hapten–lecithin mixture in the weight ratio of 1:3. Ganglioside (0.01 mg) completely removed all cytotoxic activity from 0.1 ml serum. Lecithin alone was ineffective. Absorption with an acetone dried rat brain powder removed both complement-fixing activity against ganglioside and cytotoxicity to cerebellar cells. The activity of the antiserum was unaffected by similar absorptions with rat liver powder. A further indication of the specificity of the reaction for ganglioside is that anti-galactocerebroside sera having high titres of complement-fixing activity against galactocerebroside–auxiliary lipid mixtures and purified myelin fractions of rat brain, was not cytotoxic for cerebellar cells.

The antigen on the cell surface seems to be a glycolipid and not a glycoprotein. After partitioning of a chloroform–methanol extract of cerebellar cells the ability to absorb out cytotoxic activity from anti-ganglioside serum was present in the water-soluble upper phase. The lower phase lipids and the insoluble residue were ineffective (Fig. 3). Absorption of antibody was dependent on the presence of the auxiliary lipid lecithin, further confirmation that we are dealing with a lipid hapten.

Ganglioside can be demonstrated in cells of the granular layer of the cerebellum of the adult rat by the immuno-peroxidase method⁹. Histochemical localisation in tissue sections, however, does not provide information on the number of cells with surface antigen. The present data suggests that in the immature animal, at least, this is in the region of 60–80%. The nature of the cell fraction

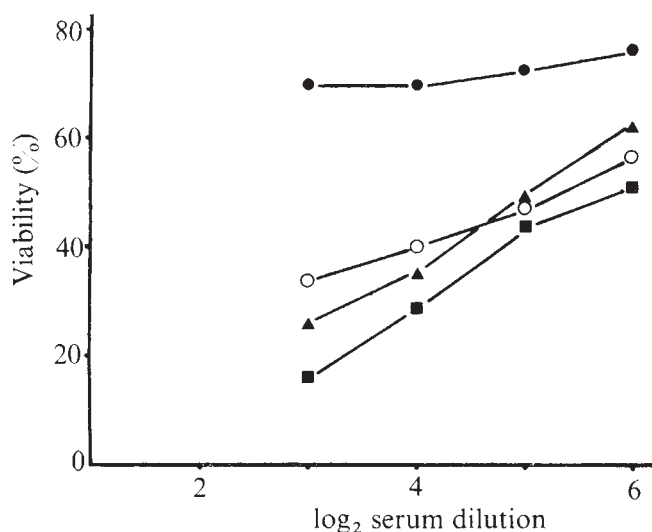


Fig. 3 Cytotoxicity of anti-ganglioside serum with fractions obtained by chloroform–methanol treatment of cerebellar cells. ●, Upper phase absorbed; ○, residue absorbed; ▲, lower phase absorbed; ■, unabsorbed serum. About 5×10^7 cerebellar cells ml^{-1} were extracted in chloroform–methanol (1:1, v/v). This insoluble residue was centrifuged and resuspended by homogenisation and ultrasonication in 0.5 ml saline. Chloroform was added to the lipid extract to make it 2:1 chloroform–methanol and then partitioned against 0.2 volume of saline. Both upper and lower phases were evaporated to dryness under N_2 . Lecithin (100 μg) was added to the upper phase. Both residues were re-suspended in 0.5 ml saline with the aid of ultrasound. One-quarter dilution of serum (0.5 ml) was added to each of the three fractions and stored at 4 °C overnight. After centrifugation the cytotoxic activity of the absorbed and a control serum for rat cerebellar cells was determined. Cytotoxicity was measured by ^{51}Cr release. Cerebellar cells were incubated with 50 μCi ^{51}Cr in saline for 0.5 h. They were diluted in 3 ml Krebs buffer and centrifuged through 4% bovine serum albumin in Krebs buffer to obtain labelled intact cells. The supernatant was removed and the walls of the tube wiped before resuspending. Cell suspension (100 μl), serum (50 μl) and guinea pig complement (50 μl) were incubated for 1 h at 37 °C. The reaction was stopped in an ice bath and 1.0 ml ice-cold medium added. After centrifugation 0.6 ml supernatant was removed and the radioactivity in supernatant and infranatant counted.

$$\% \text{ Viability} = \frac{\frac{\% \text{ Cr release (serum + complement)}}{\% \text{ Cr release (in water)}} - \frac{\% \text{ Cr release (complement alone)}}{\% \text{ Cr release (complement alone)}}}{\% \text{ Cr release (in water)}} \times 100$$

lacking surface ganglioside is unknown. It may constitute a particular cell type or merely reflect the immaturity of a proportion of cerebellar cells at this stage of development. The maximal rate of ganglioside increase in the developing rat brain occurs 10–20 d postnatally, although increased synthesis can be detected earlier—before the onset of myelination. The enzyme glucosyl transferase, which is thought to be involved in ganglioside synthesis, reaches maximal activity by day 7 (ref. 10).

The demonstration that at least some of the ganglioside in brain cells is accessible to antibody at the cell surface suggests that immunohistochemical methods may profitably be applied to localise these haptens on the cell membrane. It also raises the possibility that gangliosides may furnish a range of cell-surface markers for the identification and separation of cell types in the central nervous system.

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Ciliary dyskinesia factors in cystic fibrosis and asthma

CYSTIC fibrosis (CF) homozygotes and heterozygotes carriers for CF harbour a factor in their serum (termed CDF) which causes ciliary dyskinesia when applied to ciliated epithelial tissue derived from rabbit trachea^{1–4}. Spock *et al.*¹ reported that serum ciliary dyskinesia activity (CDA) was a specific marker for the CF gene. Conover *et al.*⁵ have reported that a CDA is also found in sera from individuals with various respiratory and autoimmune disorders; they have since suggested that the CF-ciliary dyskinesia factor was C3a (anaphylatoxin), and proposed that a defect or deficiency in the anaphylatoxin inactivator was the primary gene defect in cystic fibrosis^{6,7}. Other laboratories have been unable to detect CF-CDF using a rabbit tracheal bioassay for the detection of the CF-CDF (refs 8–10). Using a modified rabbit tracheal bioassay, we have confirmed that serum from individuals with bronchial asthma and serum from homozygotes and heterozygotes for CF cause ciliary dyskinesia, whereas normal healthy control sera do not. Most individuals with asthma, however, were also found to have serum which causes a subsequent ciliostasis. All sera tested by bioassay for CFP by electrofocusing indicated that all CDA-positive sera except sera from seven of eight individuals with asthma were also positive for cystic fibrosis protein (CFP). The CFP is a small (molecular weight 5,000–9,000), positively charged compound and is found in sera from most homozygotes and heterozygotes for CF (refs 11–16). These two findings suggested that the CDAs in CF sera and in asthmatic sera could be due to two different substances. We have developed two simple chromatographic techniques which can separate CDAs in CF and asthmatic sera. Our results indicate that they may be useful in purifying a CF-specific CDA. Purification of this CF-CDA is a prerequisite for its biochemical characterisation and for elucidating its role in the pathophysiology of CF (ref. 17).

Our rabbit tracheal bioassay⁴ involved tissue selection according to the criteria of Conover *et al.*², and bioassay following the protocol of Spock *et al.*¹ with minor modifications. The assay can reliably detect a ciliary dyskinesia activity (CDA) in sera from both heterozygotes and homozygotes for CF without fractionation or concentration of the serum^{1,4}. Unlike the assay of Conover *et al.*², all sera can be screened at 27 °C. Prewarming the serum to 37 °C before bioassay increased the kinetics of the reaction sufficiently to allow the determination of CDA-positive individuals in 35 min or less. Using our methods, 16 CF homozygote sera reacted in 14 ± 8 min (mean ± s.d.) and 15 OHCF sera required 25 ± 8 min, whereas 13 of 14 normal healthy control sera failed to react by 60 min (the end of the assay period⁴). Our results using whole serum for analysis confirm those of Spock *et al.*¹ and Conover *et al.*² in that sera from normal healthy donors does not elicit a ciliary dyskinesia reaction, whereas both homozygotes and heterozygotes for CF apparently do harbour a CDA.

To evaluate the specificity of the CDA detected, we bioassayed serum from eight patients with bronchial asthma^{4,18}. In agreement with Conover *et al.*, we found that all eight asthmatic sera caused a ciliary dyskinesia reaction; however, sera from asthmatics generally caused a subsequent ciliostasis (cessation of ciliary beating). Concurrent evaluation of all sera for CDA by bioassay and for CFP by electrofocusing showed that all CDA-positive sera except seven of the eight asthmatic sera were also positive for CFP (our specific marker for the CF gene).

These two findings indicated that the activity detected in patients with asthma and other respiratory diseases⁵ could be a

substance different from a CF-specific CDF. To test our hypothesis we set out to purify the CDAs in asthmatic, CF homozygote and OHCF sera. We found two simple chromatographic procedures for separating and therefore distinguishing between the CDA found in CF and asthmatic sera. The first procedure involves fractionating whole serum on DEAE-cellulose (DE-52, Whatman) using 0.005 M Tris-HCl buffer, pH 8.6, and a two-step NaCl gradient (0.00 M NaCl, 0.05 M NaCl) for elution. The second procedure involves fractionation of whole serum by Sephadex G-200 (Pharmacia) gel filtration, using 0.01 M phosphate, 0.15 M NaCl buffer, pH 8.0, for elution¹⁸.

For DEAE-cellulose chromatography, 2.0 ml of serum is dialysed overnight at 4 °C in Spectrapor 3 dialysis membrane tubing (Spectrum Medical). Spectrapor 3 tubing has a known molecular weight retention of 3,500 and will effectively retain CFP, CF-CDF, and C3a if free in solution^{7,12}. The serum is then applied to a column pre-equilibrated with eluting buffer. An initial IgG-containing fraction (Fraction DEAE-I) is obtained by elution with Tris-HCl without NaCl. This fraction (DEAE-I) contains only the most cathodally migrating gamma-globulin fraction of serum [isoelectric point (pI) 8–9 (refs 18, 19)]. The column is then eluted with Tris-HCl plus 0.05 M NaCl, and a second IgG-containing fraction (plus contaminants) is eluted (DEAE-II). Both DEAE fractions are lyophilised, reconstituted to 2.0 ml, and dialysed against PBS (0.01 M phosphate, 0.015 M NaCl, pH 7.2) in preparation for bioassay.

For Sephadex G-200 chromatography, 2.0 ml of serum is fractionated and the effluent is separated into six fractions (Fig. 1). Each fraction is then prepared for bioassay as described for DEAE-I and II.

Representative results (Table 1) clearly indicate that the asthmatic CDA is found in DEAE-II and Sephadex G-200 Fraction II, whereas the CDA found in CF and OHCF sera elutes in DEAE-I and Sephadex G-200 Fractions III and IV (weak CDA). Our CFP detected by electrofocusing is also found in DEAE-I and Sephadex G-200 Fraction III^{12,18,19}. Fractionation of serum from a heterozygote carrier (CFP positive) with bronchial asthma confirmed the existence of two distinct activities in asthmatic and CF sera, as this serum had activity in DEAE-I and II and in Sephadex G-200 Fractions II, III and IV (Table 1).

We conclude that the CDAs in asthma and CF are distinct substances. Our findings may facilitate purification of what we feel is a CF-specific CDF from CF and OHCF serum. Purification is a prerequisite to elucidating the exact nature of this substance and its role in the pathophysiology of CF.

Most recently, we have found that an 850-fold purified preparation of the CDF in asthmatic serum reacts with antisera to human C3a, whereas a 650-fold purified active preparation of CF-CDF (at the same protein concentration) does not^{18,19}. We feel the different reactivities with anti-C3a antiserum further distinguish the asthmatic and cystic CDAs.

Of related interest, are the results of Bowman *et al.*^{20,21} concerning the specificity and identification of a ciliotoxic factor (CTF) found in serum from homozygotes and heterozygotes for

Fig. 1 Fractionation of 2.0-ml samples of human sera by gel filtration on Sephadex G-200. Fractions (2.0 ml) were collected at a flow rate of 5 ml h⁻¹. V₀, V_T: void volume and total column bed volume. HSA: human serum albumin.

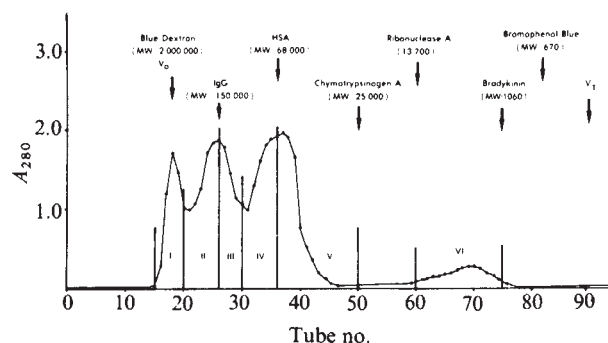


Table 1 Separation of ciliary dyskinesia activity by chromatography

Serum	DEAE-cellulose fraction		Sephadex G-200 fraction					
	I	II	I	II	III	IV	V	VI
Normal	—	—	—	—	—	—	—	—
CF homozygote	CDA*	—	—	—	CDA	CDA	—	—
Obligate heterozygote carrier	CDA	—	—	—	CDA	CDA	—	—
Asthmatic	—	CDA	—	CDA	—	—	—	—
Asthmatic-heterozygote carrier	CDA	CDA	—	CDA	CDA	CDA	—	—

*CDA: fraction produced ciliary dyskinesia by 35 min.

CF. This factor is now termed 'cystic fibrosis mucociliary inhibitor' (CFMI). CTF is detected using an oyster gill ciliary bioassay²². The investigators claim that CTF is specific for the CF gene since sera from asthmatic, allergic and normal healthy control subjects do not harbour a CTF. Although this CTF has a molecular weight, cationic charge and other properties which resemble those described for the CDF of Conover *et al.*^{6,7}, Barnett and Bowman²¹ claim that the CTF is not C3a either.

Although the identity of CTF and the CF-specific CDF described here are unknown, neither our studies nor those of Bowman and co-workers^{20,21}, lend support results^{6,7} which suggest that the CF-CDF is C3a. It remains possible, however, that the elusive 'CF factor(s)' could be related to some other component found in the complement, kinin²³, or fibrinolytic systems.

They could be, for example, abnormal fragments of C3a, C5a or other active agents which affect membrane permeability or ion transport (that is, polyamines or their aminoaldehyde derivatives²⁴⁻²⁶). We have recently shown that α_2 macroglobulin (α_2 M) is abnormal in CF and have hypothesised that this abnormality can explain the presence of 'CF Factors' (whatever their exact biochemical nature may be) in various body fluids of CF patients due to defective regulation of serum and cellular derived proteases by CF α_2 M. In addition, a defective α_2 M (possibly coupled with an abnormality in polyamine metabolism) may be responsible for the general pathophysiology of this disease²⁴⁻²⁶. Our hypothesis and findings concerning α_2 M have recently been confirmed by Shapira *et al.*²⁷.

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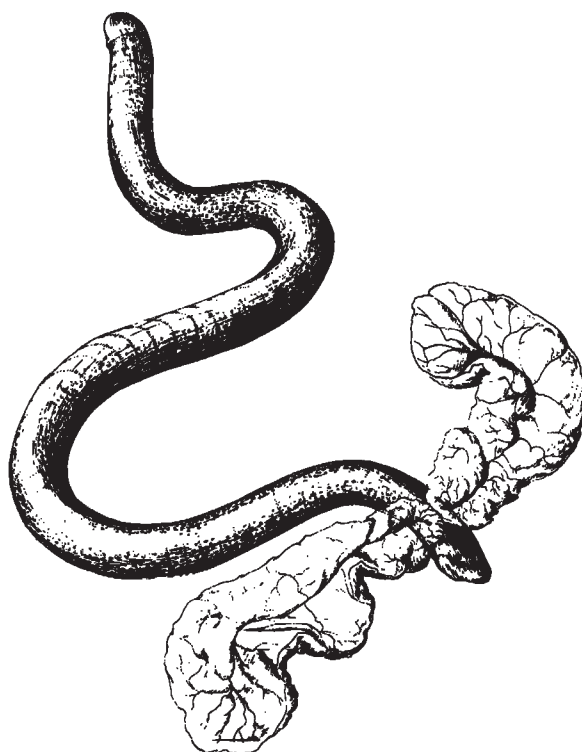
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Blood respiratory properties of a viviparous amphibian

BURROWING apodan amphibians (order Gymnophiona) are relatively common in the Amazon River Basin of South America but the physiology of the group has been little studied. The animals are usually fossorial, living burrowed in the mud banks of small tributaries of the main river. The burrows, which they presumably dig, are small and conditions are often both hypoxic and hypercarbic. Animals 0.25–0.75 m long and weighing 100–600 g, found near the air–water interface, were dug out of the mud banks and immediately taken to the laboratory of the research vessel RV Alpha Helix for study. Several species of burrowing apodans occur in the area, but the most common is *Typhlonectes compressicauda*, the animal used in this study. We report here on the blood respiratory properties of this organism.

Most female *T. compressicauda* captured had uteri containing several gilled larvae. The gills are large well vascularised jelly-like structures (Fig. 1) and the gill surfaces make close contact with the internal lining of the uterus. The structural relationship between larvae and parent in *Typhlonectes* clearly suggested a maternal supply of oxygen, and oxygen equilibrium curves were therefore constructed using pooled blood from three pregnant adult females and 27 larvae removed from several animals. Whole blood was

Fig. 1 Gilled larva removed from the uterus of a female *Typhlonectes compressicauda*, a viviparous amphibian.



used in all determinations and the technique of Edwards and Martin in curve construction was used.

Adult blood at a P_{CO_2} of 11.4 torr showed a sigmoid oxygen equilibrium curve with a P_{50} of 22.2 torr (Fig. 2). Increasing the P_{CO_2} to 17.1 torr increased the P_{50} slightly to 22.6 torr, indicating no appreciable Bohr shift. The absence of a Bohr effect would be physiologically advantageous to these animals living in small mud burrows in which the ambient P_{CO_2} fluctuates extensively. A Bohr effect in these conditions might result in the undesirable situation of facilitating oxygen unloading at the skin.

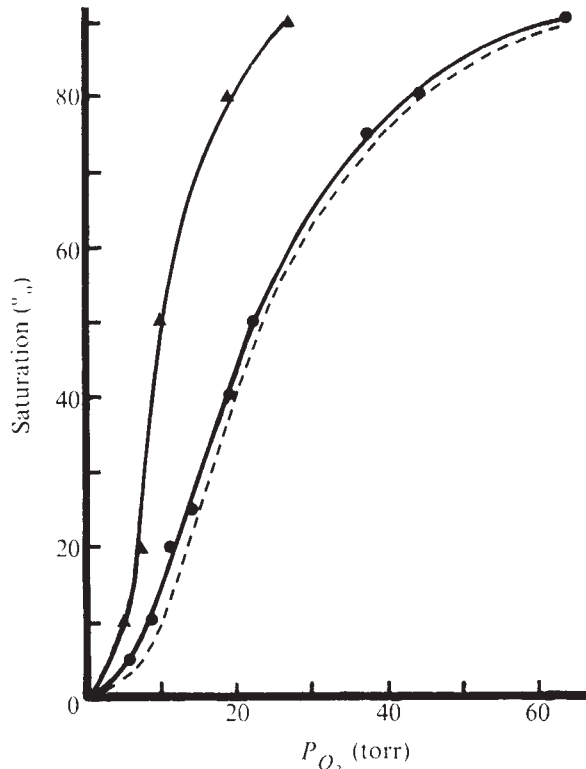


Fig. 2 Oxygen equilibrium curves of *T. compressicauda*. $\blacktriangle$, Foetal curve from gilled larvae removed from uterus P_{CO_2} 11.4 torr. $\bullet$, Maternal curve (P_{CO_2} 11.4 torr). Dashed line, maternal curve (P_{CO_2} 17.1 torr).

The foetal blood had an O_2 equilibrium shifted markedly to the left of the maternal blood (P_{50} 9.3; P_{CO_2} 11.4 torr). This intriguing feature would assist in maternal-foetal gas exchange across the uterine-gill membranes because high haemoglobin saturation of foetal blood is achieved with only moderate saturation levels of maternal blood.

Foetal-maternal shifts have been described in the viviparous garter snake *Thamnophis*² the ovoviviparous dogfish *Squalus*³ and the oviparous teleost *Scorpaenichthys*³, while Riggs⁴ has shown bullfrog tadpole curves shifted to the left of the adult. We believe our report to be the first description of a foetal-maternal shift in the Amphibia.

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One-to-one binding of a purified scorpion toxin to Na channels

DETAILED elucidation of the molecular organisation which controls ionic flow through the excitable membranes has been prevented by a difficulty in isolating substances to which characteristic features of the ionic channels can be attributed¹⁻³. In the study of the cholinergic receptor, polypeptide α -toxins from certain snakes have been successfully used for the identification and purification of nicotinic receptors⁴. Thus, agents analogous to snake toxins may be of a decisive value in the molecular approach to ionic channels, and toxins from certain scorpions are among the most promising candidates⁵⁻⁸. Scorpion toxins seem to act by modifying kinetic properties of Na channels as well as by suppressing the current through K channels⁹⁻¹¹. But, no quantitative analysis was made on an electrophysiological basis to show the precise nature of the toxin-binding. We describe here a study of the effects of a toxin from *Leiurus quinquestriatus* on Na, Ca and K currents in the tunicate egg membrane, where each ionic current proved to be essentially identical with that in other excitable membranes^{12,13}. The discrete critical membrane potentials for the activation of these currents in the egg facilitate discrimination of the respective currents only by adjusting potential steps in the voltage-clamp condition. Thus, quantitative aspects of the inactivation kinetics of Na current can be analysed conveniently in this preparation.

A crude toxin (Sigma) induced a slowly inactivating component of the Na current and enhanced it in a dose-dependent manner (Fig. 1, inset). The effective component was therefore fractionated on a CM-Sephadex C-25 column, taking the amount of the current remaining at a definite time after the potential step as a measure of toxic activity. Since the action of the toxin on the egg membrane was highly reversible, all recordings for the respective fractions were made with the same single preparation. Active fractions were collected, rechromatographed and finally gel-filtered on Sephadex G-50. There was substantial agreement between the toxic activity and absorbance profiles during the last filtration procedure. SDS-8 M urea gel electrophoresis¹⁴ of the isolated toxin showed a purity of >90%. Molecular weight was estimated to be 6,000 by gel-filtration on a Sephadex G-50 column.

The effect of varying concentration of this purified toxin on the Na current is illustrated in Fig. 2a. In the presence of the toxin, the current was inactivated in two phases—an initial fast phase (dotted line) and later slow phase (solid line). It is essential to note that an increase in the dose enhances the slow component with a unique time constant of 309 ± 6 ms (about 40-fold greater than toxin-free control). The slow components were subtracted from the respective original current traces and the fast components isolated analysed (Fig. 2b); the fast components had a common inactivation time constant of 8.3 ± 0.3 ms regardless of the dosage. The value was comparable with 8.5 ms obtained for the toxin-free control. The amount of this fast component was decreased as the final concentration of the toxin in the medium was increased. It was strongly suggested that the slow component is the current through Na channels modified by the binding of the toxin, while the fast one is that through intact Na channels. To analyse the dose-response profile for the toxin, the slow component corrected to the initial zero time of the potential step (I_0^* in Fig. 2a) was illustrated in a double reciprocal plot (Fig. 2c). A straight line was obtained, yielding K_D value of $0.46 \mu M$. When the reciprocal of the fast component similarly corrected to zero time (I_0 in Fig. 2b) was plotted directly against toxin concentration, a straight line was again obtained (Fig. 2d). The extrapolated value of the I_0 to toxin-free condition (intercept on the ordinate) was in precise agreement with the peak value of the toxin-free control current, while from the intercept on the abscissa the same K_D value of $0.46 \mu M$ was deduced as in the case of the slow component. Thus purified scorpion toxin binds to Na channel in one-to-one stoichiometry and modifies its inactivation kinetics. The maximum I_0^* was

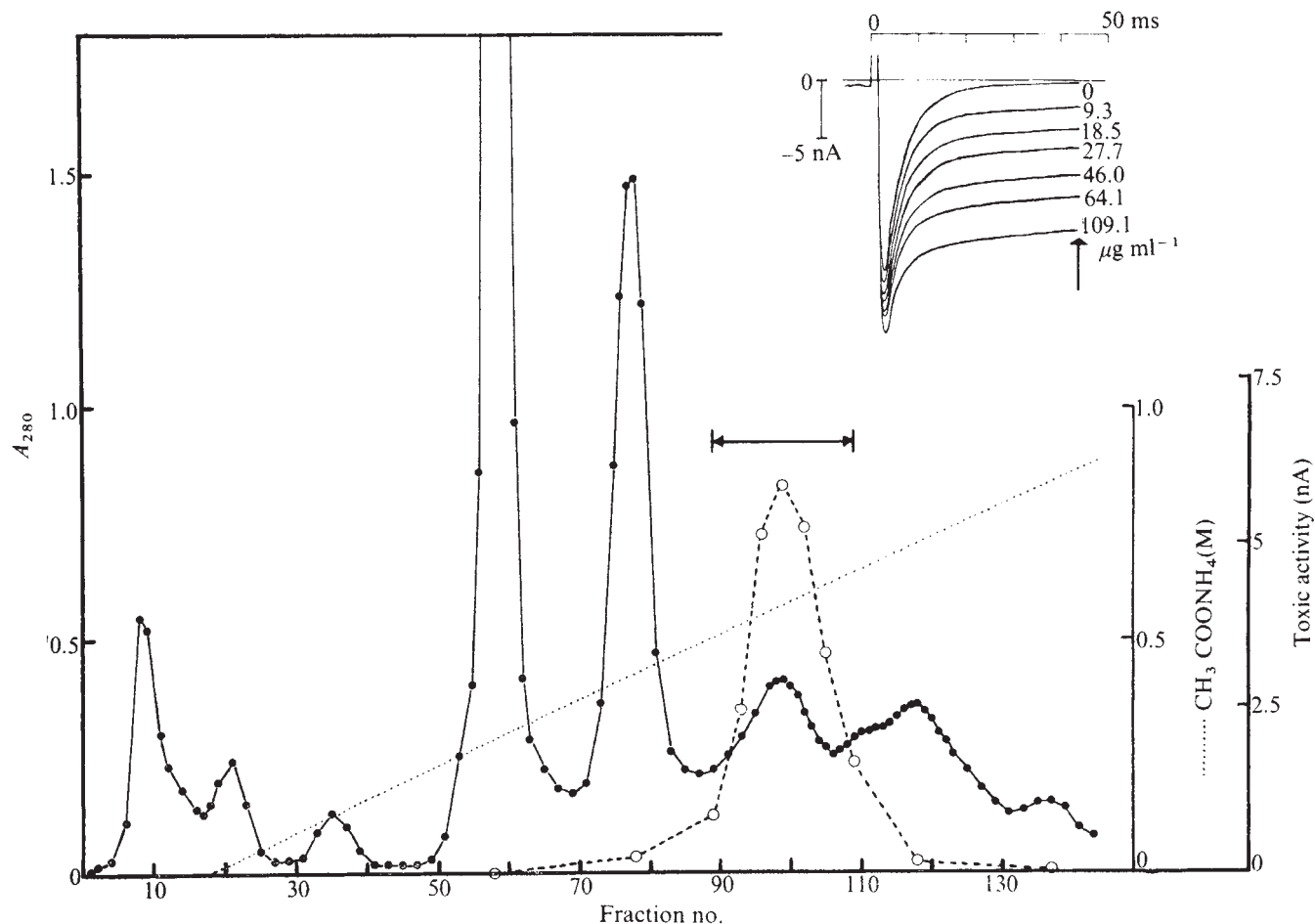


Fig. 1 Purification of a scorpion toxin on CM-Sephadex C-25 column. Venom (40 mg) from *L. quinquestratus* was extracted with 4 ml of 5 mM ammonium acetate, pH 5.5, and applied to a column (0.75 × 20 cm) equilibrated with the same buffer. The column was washed with 18.4 ml of 5 mM ammonium acetate, pH 5.5, and then eluted with a linear gradient from 5 mM (pH 5.5) to 1 M (pH 7.0) ammonium acetate (the total volume, 140 ml). Protein was monitored by absorbance at 280 nm (●). Aliquots (50 µl) of representative fractions were added to the medium (5.5 ml) bathing a tunicate egg and toxic activity was determined (○). The activity was expressed as the amount of current remaining at the indicated time (arrow in inset). Dotted line represents ammonium acetate. Inset: effect of increasing concentration of the crude venom on Na current in a tunicate egg. The methods of preparing the egg from a tunicate, *Halocynthia roretzi*, and recording the membrane currents were as described before¹². Holding potential was -90 mV and experimental temperature was 15.5 °C in this and successive figures. The test pulse in this figure was to -2 mV. Bathing medium (6.3 ml) was continuously circulated with the aid of a peristaltic pump, and small volumes of a concentrated venom solution were added successively to the reservoir until the indicated concentrations were obtained. The ionic composition of the medium in this experiment: 467.5 mM NaCl, 10 mM KCl, 50 mM MgCl₂ and 5 mM MnCl₂. Mn, instead of Ca, was incorporated in the medium to eliminate possible interference of Ca current with Na current to be measured. The medium included bovine serum albumin 4 mg ml⁻¹, and 5 mM PIPES-choline (pH 6.9) in this and successive figures.

slightly lower than that of I_0 , suggesting a reduced permeability coefficient for the toxin-modified channels (about 85% of the control).

Figure 3 shows the channel specificity in the action of the purified toxin. Li current through Na channels¹² was modified as above (Fig. 3a), but K and Ca channels were not affected (Figs 3b, c). Further, a detailed analysis indicated that the toxin exerts little, if any, influence on the activation kinetics of Na channels.

Reduction of the cationic concentration in the medium led to a decrease in the K_D value of the toxin-binding. When the ratio of unmodified (I_0) to modified (I^*) currents at varying external Na concentrations and at a constant level of toxin was plotted against $[Na]^2$, a linear relation was obtained (Fig. 2e). Since this ratio is expected to be proportional to the K_D value in case of one-to-one binding, the competitive inhibition of the toxin-binding by Na ions was indicated. Essentially similar results were obtained with Li, Ca, Mn and La ions in the medium. This suggests that there are multiple binding sites for cations; the cooperativity of these sites may differ to a certain extent depending upon the ionic species to be bound, because Hill coefficients varied around 2 according to the species of cations tested.

Our results clearly demonstrate, on an electrophysiological basis, that a toxin purified from venom of *L. quinquestratus* specifically binds to Na channels in the tunicate egg membrane and modifies almost exclusively the inactivation kinetics. Electrically excitable neuroblastoma cells have been shown to contain a similar

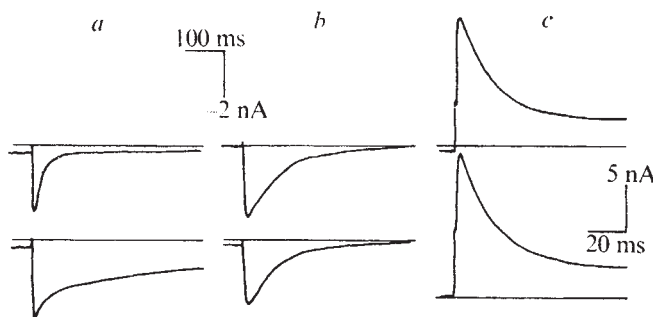


Fig. 3 Channel specificity in the action of a purified scorpion toxin on the tunicate egg membrane. *a*, Li currents through Na channels in the absence (upper trace) and presence (lower trace) of 2.5×10^{-8} M purified toxin. Test pulse to -22 mV. Ionic composition of the medium—50 mM LiCl, 5 mM MnCl₂ and 5 mM KCl. Tonicity was adjusted by the addition of glucose. *b*, Ca currents were obtained by a test pulse to +27 mV with a prepulse to -25 mV to inactivate the Na current¹². The durations of the prepulse were 0.4 s in the absence of toxin (upper trace) and 6.0 s in its presence at 1.7×10^{-6} M (lower trace). Ionic composition—400 mM NaCl, 10 mM KCl, 50 mM CaCl₂ and 50 mM MgCl₂. *c*, K currents were activated by a test pulse to +125 mV with a prepulse to -37.5 mV (ref. 12). The durations of the prepulse were 0.4 s in the absence of toxin (upper trace) and 10 s in its presence at 0.47×10^{-6} M (lower trace). Ionic composition was the same as in Fig. 1. In *b* and *c*, it was confirmed that the toxin effectively modified the inactivation kinetics of Na current.

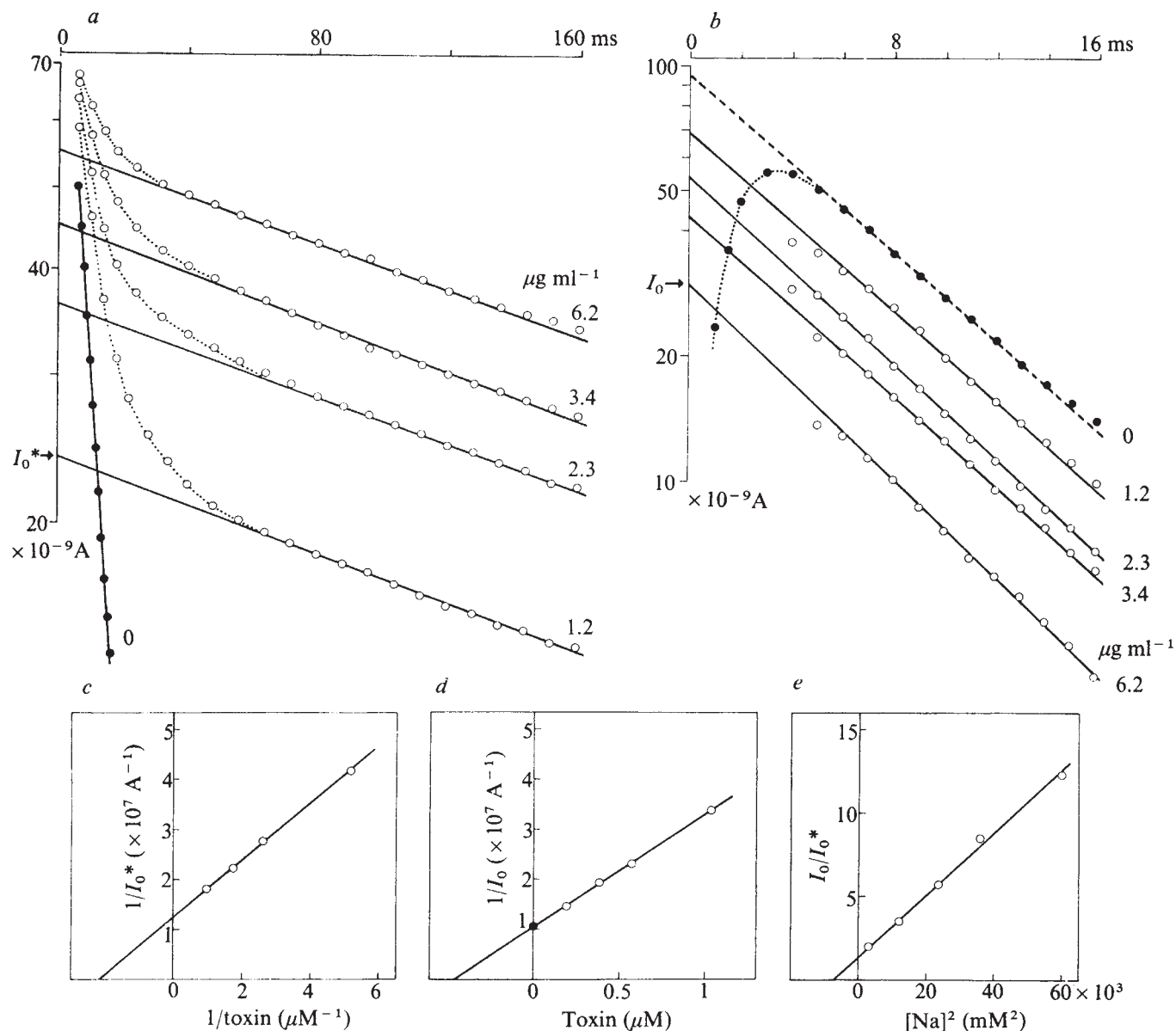


Fig. 2 Effect of a purified toxin on inactivation process of the Na current. *a*, Na currents were measured as described for Fig. 1, except for the test pulse to -9.5 mV. Purified toxin was added successively until the indicated concentration was obtained. The logarithm of the current in the presence ($\circ$) or absence ($\bullet$) of the toxin was plotted against time. *b*, The slow components (solid lines in *a*) were subtracted from the respective original currents and the logarithms of the fast components isolated were plotted against time ($\circ$). For a comparison, the Na current in toxin-free medium was also included ($\bullet$). *c* and *d*, the reciprocals of the slow and fast components corrected to zero time (I_0^* in (*a*) and I_0 in (*b*)) were plotted against the reciprocal of toxin concentration (*c*), and against toxin concentration (*d*), respectively. *e*, Inhibition of toxin-binding by Na ions. External Na concentration was successively increased at a constant level of the toxin (1.7×10^{-8} M) and with 5 mM MnCl_2 and 5 mM KCl . MgCl_2 was omitted and tonicity was adjusted by the addition of glucose. The ratio of I_0 to I_0^* at the indicated concentration of Na was calculated as in (*a*) and (*b*), and was plotted against $[\text{Na}]^2$. The Na currents used for this estimation were obtained with a test pulse to -20 mV.

single class of binding sites for the toxin from *L. quinquestratus*⁷. In this experiment the active toxin had been isolated by monitoring its stimulative effect on the tetrodotoxin-sensitive ^{22}Na influx induced by the simultaneous presence of veratridine, whereas our purification procedure was based directly on the electrophysiological effect of the toxin. Although the purified toxin may be identical in these two experiments, the reported K_D values differ widely (1.3–2.4 nM in neuroblastoma and 460 nM in the tunicate egg). This apparent discrepancy, however, can largely be attributed to a higher cationic concentration in the artificial sea water used in our experiment. In fact, the K_D of about 10 nM was estimated in the tunicate egg in a cation-free condition, taking the competitive inhibition by cations in the medium into consideration. It is likely that the presumptive inactivation particles of Na channels¹⁵ bear multiple sites that bind various cations, and these sites may be blocked by a single scorpion toxin molecule to result in the retardation of the inactivation gating.

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Does intracellular sodium modify membrane permeability to sodium ions?

In sodium transporting epithelia the asymmetrical cells have the apical face specialised for sodium entry while the basolateral membranes are concerned with sodium export, the accompanying anion passing passively. Epithelia exposed to various sodium concentrations may need to control sodium entry as a way of regulating the intracellular concentration. Some amphibian epithelia can show adaptive changes in their transporting capacity with changes in the ambient sodium concentration¹⁻³. In addition, rapid changes in permeability of the apical face occur with time constants of a few seconds when epithelia are exposed to step changes in sodium concentration⁴. While the slow adaptive changes may involve alterations in synthesis of membrane macromolecules important for membrane permeability, the rapid changes are likely to result from fast changes involving the existing membrane apparatus. One effector agent for the rapid reactions may be the intracellular sodium concentration and the experiments reported here were designed to test this hypothesis. We have manipulated frog skin epithelium in ways to increase the intracellular sodium content and found that the density of sodium channels in the apical face, measured by labelling with ¹⁴C-amiloride, is reduced in these circumstances.

¹⁴C-amiloride has been used previously to label sodium channels in frog skin and isolated toad bladder cells^{5,6,7}, and the amount of ¹⁴C-amiloride displaced from binding sites by unlabelled drug when the former is used at a concentration of 10 nM in the presence of 1.1 mM Na⁺ is a measure of binding to sodium channels. As external sodium is reduced the apparent affinity of amiloride increases⁸. Labelling is carried out with only low concentrations of sodium present to minimise the concentration of ligand required, and so reduce nonspecific binding.

Part of the evidence that amiloride binds at, or close to, sodium entry sites is given by the correlation of site density and the level of sodium transport which can be maintained by the tissue. Epithelia deprived of sodium for 1 week have been found to have a threefold increase in site density and can, when re-exposed to sodium, maintain a transport level three times that of control skins³.

We measured ¹⁴C-amiloride binding in the mucosal surface of frog abdominal skin (*Rana temporaria*, area 9.6 cm²) as described before^{3,5,6,8}. To increase sodium concentration on the inside of the apical membrane the serosal surface was treated with ouabain or K-free solution to reduce or abolish the activity of the sodium pumps. Throughout the experiments the transepithelial voltage was maintained at zero by an automatic voltage-clamp device.

In each experiment, five measurements were made of the amount of displaceable amiloride bound to the skin. Subsequently, a further five measurements were made after ouabain had been used to reduce transport to a low level (about 90% inhibition).

Results of experiments in which amiloride binding was measured in the presence of 1.1 mM Na⁺ are given in Table 1. After transport had been inhibited by ouabain there was no significant difference in the amount of amiloride bound. In these experiments, and before ouabain inhibition, the intracellular sodium concentration was in a steady state, in which sodium influx through the mucosal face was equal to sodium efflux through the serosal face. After ouabain, it is unlikely that the intracellular sodium con-

centration increased as the mucosal concentration was only 1.1 mM, and furthermore, the favourable electrical gradient for sodium entry could only decrease after sodium pump inhibition. Sodium can enter and leave the cells only by the mucosal face after pump inhibition, as shown by studies with the electron probe⁹.

If the mucosal face of the skin is flushed with high sodium solution (111 mM) and then returned to low sodium (1.1 mM) conditions before binding is measured, the increased intracellular sodium concentration is lowered rapidly, mainly by pumping through the serosal surface. After ouabain, however, this exit route will be blocked. Experiments such as those described in Table 1 were repeated except that each binding measurement was preceded

Table 1 Effect of ouabain on amiloride binding in low sodium solution

	Control	After ouabain (10 ⁻³ M)
Total binding (10 nM ¹⁴ C-amiloride)	1.44 ± 0.07	1.99 ± 0.05
Non-displaceable binding (10 nM ¹⁴ C-amiloride plus 1 μM unlabelled amiloride)	1.20 ± 0.07	1.76 ± 0.05
Displaceable binding	0.23 ± 0.03 (P < 0.001)	0.20 ± 0.04 (P < 0.001)
Δ = 0.03 NS		

Amiloride binding is expressed as pmol per 9.6 cm². The concentration of sodium was 1.1 mM. Means and s.e. are from results for 15 determinations in three separate skins. The significance of the specific (displaceable) binding was estimated using a paired *t* test. The serosal surface was bathed in Ringer's solution (mM; NaCl, 111; CaCl₂, 1; KCl, 2; Tris, pH 7.6, 5 and glucose 11.0). The mucosal solution contained only 1.1 mM NaCl and no glucose. ¹⁴C-amiloride had a specific activity of 54 Ci mol⁻¹. NS, not significant.

by a high sodium flush. The results (Table 2) show that inhibition of transport with ouabain reduces amiloride binding to 36% of its control value. If the equivalence between amiloride-binding sites and sodium channels is accepted, the result after ouabain corresponds to a decrease in the mucosal sodium permeability, effected by a reduction in availability of channels. An ouabain-like effect was also obtained when potassium was omitted from the serosal bathing solution; this produced a 31% reduction in channel density.

So far, our results indicate that an increase in intracellular sodium (or possibly a decrease of intracellular potassium) reduces amiloride binding, and therefore the availability of sodium channels, in the mucosal face of the cells. This may not, however, be due to intracellular sodium changes *per se*, but to other effects, such as a potential change across the mucosal membrane. For example, changes in transepithelial potential, *V_T*, change the extent of amiloride binding⁶. This could be clarified if it were possible to control the potential across the mucosal membrane,

Table 2 Effect of ouabain on amiloride binding in low sodium solution following a high sodium flush

	Control	After ouabain (10 ⁻⁵ M)
Total binding (10 nM ¹⁴ C-amiloride)	1.72 ± 0.10	2.02 ± 0.07
Non-displaceable binding (10 nM ¹⁴ C-amiloride plus 1 μM unlabelled amiloride)	1.47 ± 0.09	1.93 ± 0.07
Displaceable binding	0.25 ± 0.03 (P < 0.001)	0.09 ± 0.04 (P < 0.02)
Δ = 0.16 P < 0.005		

Amiloride binding is expressed as pmol per 9.6 cm². Concentrations of sodium were 1.1 mM (low) and 111 mM (high). Means and s.e. are from results for 20 determinations in four separate skins. Ringer's solution was used to flush the mucosal surface before returning to low sodium solution to measure binding.

V_m , rather than across the whole epithelium. To achieve this we have used the potassium depolarised preparation of Morel and Leblanc^{10,11}. The serosal side of the skin was bathed in a potassium-rich solution so that the resistance and potential across the serosal border are reduced to low values¹². In this situation the transepithelial resistance is located mainly at the mucosal membrane, presumably making it possible to clamp this membrane at, or close to, zero. K-depolarised skins are able to maintain low level transport, and are still sensitive to amiloride and ouabain^{10,11}.

Table 3 gives the results obtained with K-depolarised skins. Again, amiloride binding was measured in low sodium conditions immediately after a high sodium flush. After ouabain had been used to inhibit serosal efflux, the amount of binding was decreased by 64%. It seems, therefore, that in this situation the reduction in binding could not have been entirely due to potential changes, but was more likely due to increased intracellular sodium.

At this stage it was necessary to check that ouabain did not cause a change in affinity of amiloride for the binding site so as to lead to reduced binding. Apparent affinities for amiloride (reciprocal of concentrations producing 50% inhibition of transport) were determined in six control skins bathed on both sides with Ringer's solution. The mean value (520 nM) fell within the normal range. Ouabain was then used to reduce sodium transport to about 30% of its control value. The affinity was redetermined on the residual sodium currents. No significant difference in apparent affinity was found after ouabain. We concluded that the changes in binding caused by ouabain were not the result of changes in the affinity of the ligand for the binding sites.

Table 3 Effect of ouabain on amiloride binding in low sodium solution after a high sodium flush with the serosal surface bathed in high potassium solution

	Control	After ouabain (10^{-3} M)
Total binding (10 nM 14 C-amiloride)	1.35 ± 0.06	1.73 ± 0.05
Non-displaceable binding (10 nM 14 C-amiloride plus 1 μ M unlabelled amiloride)	1.10 ± 0.08	1.63 ± 0.06
Displaceable binding	0.25 ± 0.03 ($P < 0.001$)	0.09 ± 0.02 ($P < 0.005$)
$\Delta = 0.16$ $P < 0.005$		

Amiloride binding is expressed as pmol per 9.6 cm^2 . Concentrations of sodium were 1.1 mM (low) and 111 mM (high). Means and s.e. are from results for ten determinations in two separate skins. The serosal bathing solution was Ringer's in which all the NaCl was replaced with KCl.

The mean value for specific amiloride binding in control conditions in the experiments reported here was $0.243 \text{ pmol per } 9.6 \text{ cm}^2$. This corresponds to 153 binding sites per μm^2 at an occupancy of 30–40% with 10^{-8} M amiloride, or about 400 binding sites per μm^2 at 100% occupancy, a value comparable with those reported elsewhere^{3,5–8}.

Our results provide a direct demonstration that an increase in intracellular sodium is accompanied by a reduction in the availability of amiloride-binding sites, and presumably of sites through which sodium entry can occur. This finding provides a mechanism to explain why the measured sodium influx through the mucosal face is inhibited by ouabain^{11,13} and shows that reduced influx is not simply a result of a reducing gradient as the transport pool fills up. When both sodium and amiloride are present in the external solution they show competition, perhaps at a site which needs to be occupied for sodium ions to be translocated through the channel. Intracellular sodium seems to make a fraction of the channels unavailable to external Na^+ or amiloride, thus reducing the transepithelial current, possibly by interaction at a regulator site different from the site for translocation. The slow time course of the effect of step changes in external sodium concentration⁴ might indicate that this too affects transport by increasing intracellular sodium.

The simple feedback operated by intracellular sodium may be an

important homeostatic device for the transporting cell. For example, with conditions where the supply of energy for the pump is impaired, the intracellular sodium content may be kept low. There is increasing evidence that intracellular sodium determines levels of intracellular calcium by a control exerted at the mitochondria^{14–16}. Increases in intracellular calcium may well be unacceptable in a tight epithelium if they lead to a loss of cell coupling¹⁷.

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Endogenous C-type viral expression in primates

C-TYPE viruses are genetically transmitted in species representing several orders of vertebrates (for review see refs 1 and 2). So far the isolation of endogenous viruses of primates has been limited to species of *Papio*^{3–5}, although related viral nucleic acid sequences have been detected in cellular DNAs of several other Old World monkeys⁶. The extent of divergence of known endogenous primate viruses should be useful in predicting properties of the gene products of evolutionarily related endogenous viruses of primates from which such viruses have yet to be isolated. We report here an investigation of the antigenic divergence of different structural proteins of prototype endogenous primate C-type virus isolates. Endogenous viral antigen expression was also examined in tissues of primate species, representing the major genera of the order Anthroidea. Immunologically divergent gene products of endogenous viruses were found to be expressed in tissues of Old World monkeys, including many species that have not yet yielded complete virus. These findings, together with the marked variations in viral antigen expression demonstrated among different primate genera, have implications for endogenous virus regulation in primates and the search for related viruses of humans.

The development of immunoassays for the 30,000 molecular weight major structural protein (p30)^{7,8}, the 70,000 molecular weight glycoprotein (gp70)⁸ and a 15,000 molecular weight protein (p15)⁹ of a prototype virus isolate of *P. cynocephalus* made it possible to compare the immunological relatedness of several endogenous primate viruses. Known C-type viral proteins contain a range of antigenic determinants that vary in their degree of cross-reactivity with analogous proteins of viruses of other species. In competition immunoassays using a given ¹²⁵I-labelled viral protein with limiting antiserum prepared against the same virus, the extent to which another virus can compete reflects the immunological relatedness of analogous proteins of the two viruses. As Fig. 1 shows, *P. cynocephalus* virus competed with high efficiency and to a final extent of more than 95% in immunoassays for

three of its structural proteins. Endogenous viruses of *P. hamadryas* and *P. anubis* reacted as efficiently as *P. cynocephalus* virus in the immunoassay for *P. cynocephalus* virus in the immunoassay for *P. cynocephalus* virus p30 (Fig. 1a). In the assay for *P. cynocephalus* viral gp70, the other baboon viruses competed only as much as 80% (Fig. 1b), while neither competed more than 50% in the *P. cynocephalus* virus p15 immunoassay (Fig. 1c).

Certain endogenous viruses of the cat have been reported to be of primate origin and to have secondarily entered the cat lineage several million years ago^{10,11}. Figure 1 demonstrates greater antigenic divergence of proteins of evolutionarily related feline viruses from those of the *P. cynocephalus* virus. Endogenous viruses of *Felis catus* and *F. sylvestris* showed significantly less ability to compete than the baboon viruses in the *P. cynocephalus* virus p30 assay (Fig. 1a) and even more striking antigenic divergence from *P. cynocephalus* virus in their p15 and gp70 reactivities (Fig. 1b and c). The relative conservation of p30 antigenic determinants suggests stronger evolutionary selective pressures for maintenance of the immunological integrity of this protein. Whether this also reflects preservation of some important biological function(s) is not resolved. In any event, it can be seen that the immunoassay for *P. cynocephalus* viral p30 was most broadly-reactive and thus most likely to be useful in a search for antigenic reactivity of related viruses in primate tissues.

Previous studies have demonstrated the expression of endogenous C-type viral antigens in normal tissues of vertebrates including the mouse¹²⁻¹⁴, cat¹⁵ and rhesus monkey^{9,15}. In a systematic search for C-type viral gene products in tissues of a wide range of Old World monkeys, liver cell extracts were analysed for expression of antigens cross-reactive with *P. cynocephalus* viral p30. Cell extracts were subjected to DEAE-cellulose chromatography in an effort partially to purify p30 and reduce nonspecific reactivity⁹.

As Table 1 shows, antigens cross reactive with *P. cynocephalus* viral p30 were detectable in tissues of species representing each of five genera of Old World monkeys (Table 1). The specificity of the antigenic reactivity was indicated by lack of competition in immunoassays for the p30s of other mammalian C-type viruses, including mouse and woolly monkey isolates (data not shown). Partially purified p30 antigens were subjected to molecular size analysis by gel filtration chromatography in both denaturing (6 M GuHCl) and non-denaturing conditions. In each case, tissue-associated p30 reactivity chromatographed with a molecular weight of about 30,000 relative to standards (data not shown). This sizing step generally provided a further 8-10-fold purification (Table 2). The degree of purification was sufficient to allow detection of p30 related to *P. cynocephalus* virus at a level about 50 times lower than that present in macaque tissues. The same procedures failed to reveal evidence of similar antigenic reactivity in more than 100 individual tissues analysed from higher apes or humans (Table 1).

As Table 1 shows, species representing two primate genera, *Papio* and *Macaca*, exhibited marked differences in their tissue expression of viral p30. Macaque tissues had relatively high p30 levels, from 15 to more than 100 ng per mg cellular protein. In most cases, baboon tissues had levels at least 20 times lower. Attempts to isolate endogenous C-type viruses from macaques have been unsuccessful. Yet, in similar conditions such viruses have been obtained readily from most species of baboon (refs 3-5 and our unpublished observations). This could reflect the release from macaque tissues of viruses with very different host ranges from those of known baboon endogenous viruses. Alternatively, the high levels of endogenous viral p30 expressed by macaque tissues in the absence of detectable virus release could be due to partial expression of a defective viral genome or to a late cellular block to release of a competent virus.

In view of the highly-conserved nature of p30 antigenic

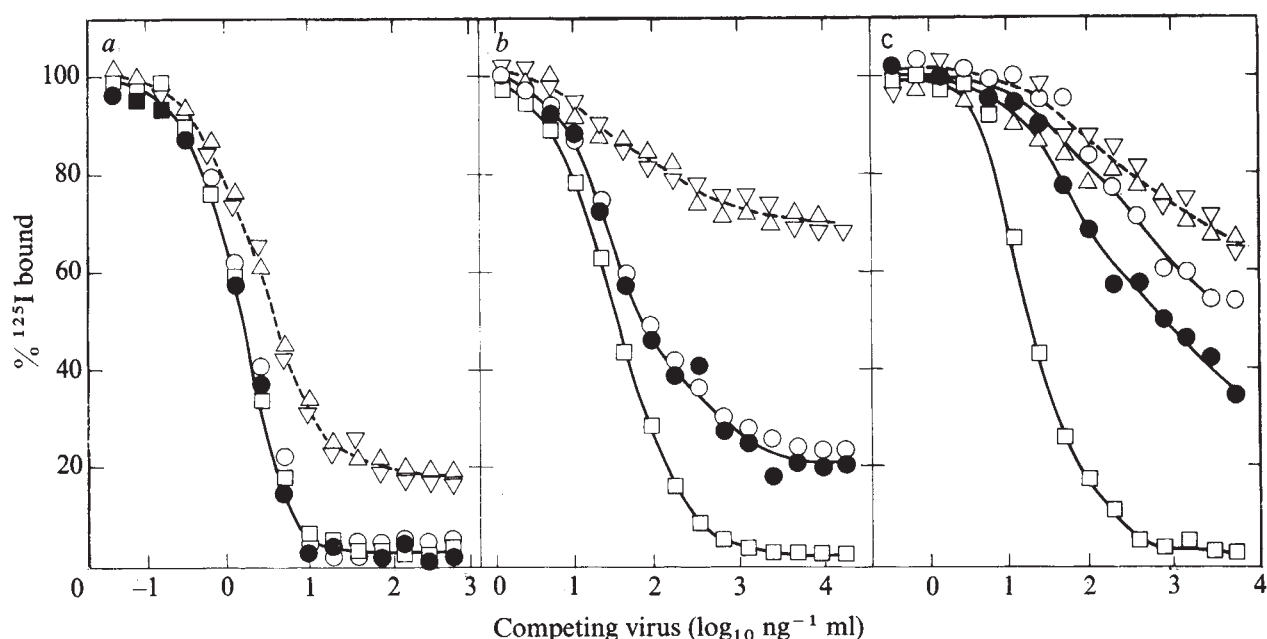


Fig. 1 Immunological divergence of viral structural proteins of endogenous primate C-type viruses. Competition immunoassays were performed as described in Table 1 and included assays in which antiserum to the *P. cynocephalus* virus was used to precipitate ¹²⁵I-labelled *P. cynocephalus* viral p30 (a), gp70 (b) and p15 (c). Viruses, concentrated from tissue culture fluids by sucrose density centrifugation, were disrupted by incubation in 1% Triton X-100 at 37 °C for 30 min. Viruses tested included isolates of *P. cynocephalus* (□); *P. hamadryas* (○), and *P. anubis* (●); and endogenous viruses of two cat species, *F. catus* (△) and *F. sylvestris* (▽). The isolation of *P. cynocephalus*³, *P. hamadryas*⁵ and *F. catus* viruses¹⁰ have been described. *P. anubis* and *F. sylvestris* viruses were isolated in our laboratory from normal kidney tissues of these species.

Table 1 Expression of antigen related to that of the major structural protein (p30) of *P. cynocephalus* virus

Family	Genus	Species	Common name	Viral p30 antigenic reactivity		
				No. tested	No. positive	Range (ng p30 per mg cellular protein)
Cercopithecoidea	<i>Cercocebus</i>	<i>C. atys</i>	Sooty white-crowned mangabey	1	0	<1
	<i>Cynopithecus</i>	<i>C. niger</i>	Celebes (black) ape	1	1	10
	<i>Papio</i>	<i>P. cynocephalus</i>	Yellow baboon	1	1	2
		<i>P. anubis</i>	Olive baboon	1	1	3
		<i>P. hamadryas</i>	Hamadryas baboon	1	0	<1
		<i>P. papio</i>	West African baboon	2	0	<1
		<i>P. sphinx</i>	Mandrill	1	0	<1
	<i>Cercopithecus</i>	<i>C. neglectus</i>	De Brazzas guenon	1	1	20
		<i>C. aethiops</i>	Griquet monkey	1	0	<1
		<i>C. patas</i>	Patas monkey	2	0	<1
	<i>Macaca</i>	<i>M. mulatta</i>	Rhesus macaque	10	10	15–110
		<i>M. arctoides</i>	Stump-tailed macaque	2	2	40–60
		<i>M. nemestrina</i>	Pig-tailed macaque	2	2	25–70
		<i>M. irus</i>	Crab-eating macaque	3	3	30–80
Pongidae	<i>Pongo</i>	<i>P. pygmaeus</i>	Orangutan	4	0	<1
	<i>Pan</i>	<i>P. troglodytes</i>	Chimpanzee	14	0	<1
	<i>Gorilla</i>	<i>G. gorilla</i>	Gorilla	1	0	<1
	<i>Hylobates</i>	<i>H. lar</i>	Gibbon ape	3	0	<1
Hominoidea	<i>Homo</i>	<i>H. sapiens</i>	Man	119	0	<1

Liver cell extracts were prepared by homogenisation in an equal volume of 0.01 M Tris-HCl, pH 7.8, 0.1 M NaCl, 0.5 mM EDTA and 0.5% Triton X-100, and sonication for 1 min with a Biosonic II sonic oscillator. Supernatants were clarified by centrifugation for 1 h at 100,000g, and subjected to partial purification by DEAE ion exchange chromatography⁸. Both original and DEAE-purified cell extracts were tested at serial two-fold dilutions for ability to compete with ¹²⁵I-labelled *P. cynocephalus* virus p30 (ref. 10) for binding limiting concentrations of goat antisera to detergent-disrupted *P. cynocephalus* virus. Antiserum and competing antigen were incubated at 37 °C for 1 h in 0.15 ml of reaction mixture containing 10 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.4% Triton X-100, 1% bovine serum albumin, 0.1% normal goat serum, 10 mM NaCl and the protease inhibitor phenylmethane-sulphonyl fluoride (300 µg ml⁻¹, Pierce Chemicals). About 10,000 c.p.m. ¹²⁵I-labelled p30 in 0.05 ml of the same buffer was added and incubation continued for 3 h at 37 °C and a further 18 h at 4 °C. Antigen-antibody complexes were precipitated by pig anti-goat IgG⁸ and radioactivity in the precipitates was measured in a Searle gamma counter Model 1285. Levels of p30 antigen in partially-purified liver cell extracts were calculated on the basis of the displacement of the competition curve for the unknown relative to that achieved with purified viral antigen. Protein concentrations were determined according to the method of Lowry *et al.*²³. Results expressed on the basis of p30 concentrations in the original extracts were determined by quantifying p30 levels in DEAE-purified extract and correcting for the degree of purification achieved.

determinants among baboon endogenous viruses, it was interesting to investigate the extent of immunological relatedness of endogenous viral p30s among other genera of Old World monkeys. As Fig. 2 shows, p30 antigens partially purified from liver tissues of *Macaca mulatta*, *Cynopithecus niger*, and *Cercopithecus neglectus*, each representing a different genus, competed less efficiently than that of p30 partially purified from *P. cynocephalus* liver tissue in the

immunoassay for *P. cynocephalus* virus p30. These findings demonstrate that endogenous viral p30s of species representing different genera of Old World monkeys have undergone sufficient evolutionary change to make them immunologically distinguishable.

The finding of gene products related to *P. cynocephalus* viral p30 in normal tissues of many species of Old World monkeys, together with previous molecular hybridisation studies⁶, indicate the widespread distribution and expression of related endogenous viral sequences in these primates. In our studies, analogous translational products were not detected in tissues of higher apes or humans. Our findings that endogenous viral p30s of other Old World primate genera can be immunologically distinguished from those of baboons indicate that p30 of an evolutionarily related human endogenous virus would probably be distinguishable as well. Although other possibilities can be considered, previous claims of the detection of p30, indistinguishable from that of *P. cynocephalus* virus in certain human tumours^{16,17}, seem to favour infection of humans with baboon C-type virus. Such a model has been invoked^{18,19} to explain the detection in certain human tumours of highly related nucleic acids^{18,19} and even infectious virus²⁰, so far indistinguishable by the present techniques from *P. cynocephalus* virus²¹. The absence of viral antigens of the baboon virus in a large number of human tissues, as reported here, suggests that productive infection with a baboon C-type virus is likely to be infrequent if it occurs at all in humans.

The demonstrated presence of endogenous viral information in many species of Old World monkeys suggests that evolutionarily-related viruses exist in higher primates as well. Reports of low levels of genetic sequence homology between *P. cynocephalus* virus and normal cellular DNAs of apes and humans⁶ would favour this possibility. The

Table 2 Partial purification of antigen related to the *P. cynocephalus* viral p30 from cell extracts of representative Old World monkey species

Genus	Viral p30 antigenic reactivity (ng per mg cell protein) after AcA54 Ultragel chromatography
<i>Macaca</i>	
<i>M. mulatta</i>	920
<i>M. arctoides</i>	610
<i>M. nemestrina</i>	700
<i>M. irus</i>	950
<i>Cynopithecus</i>	
<i>C. niger</i>	280
<i>Cercopithecus</i>	
<i>C. neglectus</i>	150
<i>Papio</i>	
<i>P. cynocephalus</i>	40
<i>P. anubis</i>	28

P30 antigenic reactivity was purified partially from liver cell extracts by DEAE ion exchange chromatography as described in Table 1. Extracts were then subjected to molecular size analysis by AcA54 Ultragel column chromatography²¹ and tested by competition immunoassay for *P. cynocephalus* viral p30 reactivity as in Table 1. Results are expressed as ng p30 per mg cell protein after purification. Immunological specificity was established by absence of competition (<1 ng per mg cell protein) in immunoassays for mouse leukaemia virus and for a C-type virus of the woolly monkey²⁵.

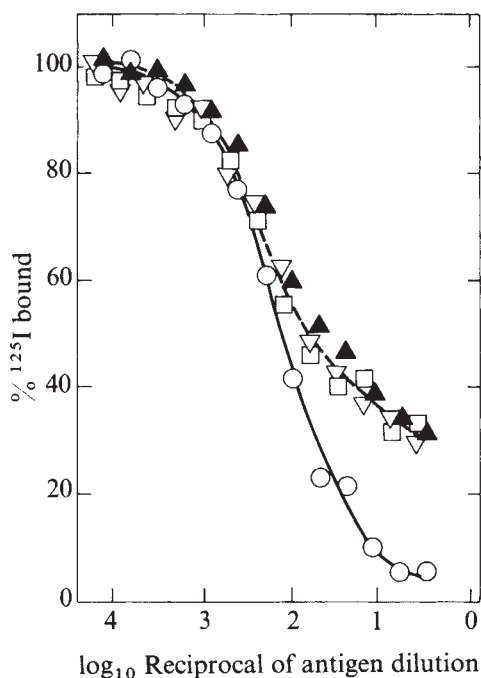


Fig. 2 Immunological divergence of endogenous viral p30 antigens partially purified from normal liver tissues of species representing different genera of the primate subfamily, Cercopitheciinae. Liver cell extracts were subjected to DEAE-cellulose ion exchange and Aca54 Ultragel chromatography before analysis in a p30 competition immunoassay utilising antiserum to the *P. cynocephalus* virus precipitate, ^{125}I -labelled *P. cynocephalus* viral p30. Reactivity of partially-purified p30s from *P. cynocephalus* (○); *M. mulatta* (□); *C. niger* (▼); and *C. neglectus* (△).

absence of antigenic reactivity related to *P. cynocephalus* virus in these tissues implies that endogenous viruses of apes and man are either very tightly restricted or have undergone sufficient antigenic divergence to have become undetectable by available immunological techniques. Knowledge that immunoassays can recognise antigenic relatedness between endogenous viruses that have diverged to the extent that nucleic acid sequence homology is no longer demonstrable²², justifies further efforts to develop more broadly-reactive and sensitive immunological probes to search for endogenous virus expression in man.

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Muscle histology and creatine kinase levels in the foetus in Duchenne muscular dystrophy

X-LINKED Duchenne muscular dystrophy is a serious genetic disorder for which at present there is no effective treatment. It is characterised by progressive muscle wasting and weakness which first becomes apparent around the age of 3–5 years, leading to death usually before the age of 20 (ref. 1). A proportion of female heterozygous carriers have significantly elevated serum levels of creatine kinase and a woman who is found to be at high risk of having an affected son can elect to have amniocentesis with antenatal foetal sexing and selective abortion of any male foetus. A proportion of such aborted foetuses will, however, be normal but since the cause of the disorder is not known it is not yet possible to diagnose an affected male foetus *in utero*. As a first step in this direction we have examined the muscle histology and levels of creatine kinase in serum, muscle and amniotic fluid in a number of therapeutically aborted male foetuses at risk for Duchenne muscular dystrophy. We report that muscle histology, and possibly the serum level of creatine kinase, may be abnormal in this disorder even by the second trimester of pregnancy.

Thirteen at-risk male foetuses were studied. In four cases (993, 1018, 75/479 and B105) the mothers were proven carriers and in the remaining cases the mothers were estimated to have a risk greater than one in five of being carriers (Table 1). In each case the sex of the foetus was established before abortion on the basis of cytogenetic studies on amniotic fluid cells obtained at amniocentesis. As controls, 13 normal male foetuses of comparable gestation were obtained at abortion performed for social reasons where there was no family history of any neuromuscular disorder. The gestational ages of the foetuses were estimated by crown-rump and heel-toe measurements².

Cryostat sections of quadriceps muscle were stained with haematoxylin and eosin. Muscle fibre diameters were measured using a calibrated Reichert Visospan projection microscope at a magnification of $\times 400$. For each foetus 200 randomly chosen fibres were measured. The percentage of hyaline fibres was determined in 500 muscle fibres. The cross-sectional areas of 100 randomly chosen nuclei, closely opposed to the surface of transversely sectioned muscle fibres, were estimated by planimetry at a final magnification of $\times 1000$. Serum specimens were obtained by cardiac puncture and serum, cell-free amniotic fluid and muscle creatine kinase activities were measured according to the method of Rosalki³. Creatine kinase activity in muscle was expressed in terms of total protein⁴.

In affected boys muscle histology is characterised by variation in muscle fibre size, fibre necrosis and phagocytosis, and ultimately replacement by fat and connective tissue. Muscle nuclear size is also increased⁵. Even in the preclinical stage of the disorder muscle histology is abnormal with hyalinisation of muscle fibres and increased variation in muscle fibre size^{6, 8}. In none of the at-risk foetuses was there any evidence of muscle fibre necrosis or phagocytosis or any increased amount of fat or connective tissue. Values outside the normal range, however, were found in six of the foetuses in regard to mean muscle fibre diameter and in five of the

Table 1 Mean muscle fibre diameter, variance in fibre size, percentage hyaline fibres, mean muscle nuclear size and serum levels of creatine kinase (SCK)

	P	Gestation (weeks)	Fibre diameter Mean (μm)	Variance (σ^2)	Hyaline fibres content (%)	Mean nuclear size (μm^2)	SCK (IU)
Normal foetuses	—	14–21	6.9–10.7	0.8–3.6	0.0–0.6	22.1–34.1	120–7350
'At-risk' foetuses							
599	0.65	21	9.7	1.4	0.0	37.1*	730
684	0.25	16	12.4	6.8	2.5	31.1*	9140
734	0.25	16	7.8	7.0	0.0	40.8*	—
993	1.00	20	7.4	1.4	0.0	33.2	—
1018	1.00	21	7.2	0.5	0.0	34.1*	1220
73/375	0.21	19	5.8	2.0	0.0	35.9*	6800
75/479	1.00	18	8.2	1.4	0.0	28.1*	1660
76/60	0.33	21	13.5	9.0	2.0	39.8*	6100
B105	1.00	20	12.4	12.3	0.5	29.0	2700
878	0.23	17	11.3	6.6	0.2	27.3	5630
76/18	0.23	20	10.9	5.6	0.0	33.4	—
B107	0.25	14	12.3	2.9	0.5	30.4	—
B109	0.25	20	10.2	2.7	0.0	31.0	1500

In normal foetuses ($n = 13$) the results are expressed as the range; in at-risk foetuses individual results are given and italicised when outside the normal range. P, probability of a mother being heterozygous. *See ref. 12.

foetuses in regard to variance (σ^2) in fibre size (Table 1). There were very few hyaline fibres in normal foetuses (0.0–0.6%) but in two of the at-risk foetuses 2% or more of the muscle fibres were hyalinised, a finding which we have noted previously in a foetus at risk for Duchenne muscular dystrophy⁹. The mean muscle nuclear size seemed to be increased in four of the at-risk foetuses.

The serum level of creatine kinase is very high in the early stages of Duchenne muscular dystrophy and is moderately raised in infancy and even at birth^{10,11}. In both normal and at risk foetuses there was considerable variation in serum levels of creatine kinase. The range in normal foetuses was 120–7350 IU and only one of the at risk foetuses (684) had a level outside this range (9140 IU). Creatine kinase levels in normal amniotic fluid were at the limit of accurate determination and no raised levels were found in any of the at-risk foetuses. Muscle creatine kinase levels were within the normal range (1.3–9.6 IU per mg protein) in all of the at-risk foetuses.

Not all of the at-risk foetuses in this study would have been expected to be abnormal. In fact from pedigree data and maternal serum levels of creatine kinase the average probability of the mothers being heterozygous was 0.51 and therefore of the 13 at-risk foetuses it might be expected that (13) (0.5) (0.51) or 3.3 might be affected. In fact, using three or more criteria of abnormality, two foetuses could be classified as being affected, one of these (684) having a raised serum level of creatine kinase. Our results suggest that X-linked Duchenne muscular dystrophy is expressed, at least in muscle tissue, by the second trimester of pregnancy.

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Degradation of deadenylated rabbit α -globin mRNA in *Xenopus* oocytes is associated with its translation

THE 3'-OH poly(A) segment of globin mRNA ensures the stability of the message when injected into *Xenopus* oocytes^{1–4}. We recently developed⁵ a specific method for the removal of the poly(A) tail from globin mRNA, based on synchronous processive phosphorolysis of mRNA using molar excess of *E. coli* polynucleotide phosphorylase at 0 °C. When injected into *Xenopus* oocytes, deadenylated globin mRNA is translated for a relatively short period and then rapidly degraded^{1,2}. Native poly(A)-containing mRNA, however, is considerably more stable in the same conditions and is translated for extended periods of time^{1,2,7}. The poly(A) segment itself is responsible for the stability of native globin mRNA in *Xenopus* oocytes since poly(A) re-addition to previously deadenylated mRNA restores the functional stability of the message³. We do not yet know, however, how poly(A) exerts its protective function; furthermore, the mechanism of degradation of poly(A)-free mRNA is not known. We report here the use of the enhancing effects of haemin on the translation of α -globin mRNA in frog oocytes⁶ to establish that, in these cells, the degradation of injected poly(A)-free α -globin mRNA is linked to its translation.

Rabbit α -globin mRNA is much less efficiently translated than β -globin message when injected into frog oocytes. The efficiency of α -globin mRNA translation can, however, be dramatically increased if haemin is included in the injection mixture⁶. The stimulation of α -globin mRNA translation by haemin is shown in Fig. 1. We took advantage of this effect to determine whether the degradation of deadenylated α -globin mRNA is linked to its translation.

We examined the effect of haemin on the degradation of poly(A)-free rabbit α -globin mRNA injected into *Xenopus* oocytes. Rabbit globin mRNA enriched in α message was prepared from the post-ribosomal supernatant of reticulocytes lysate^{8,9}; some was used as a template for the synthesis of a αH^3 -cDNA probe by a reverse transcriptase reaction¹⁰ and the remainder was deadenylated by processive phosphorolysis with polynucleotide phosphorylase⁵. Two samples of 100 oocytes from the same *X. laevis* female were micro-injected¹¹ with an aqueous solution of poly(A)-free α -globin mRNA with or without haemin. Forty oocytes from each batch were stored immediately after injection and the remaining 60 oocytes of each batch were incubated for 72 h in

Barth medium at 20 °C. We then used molecular hybridisation with the ^3H -cDNA probe to compare the amount of globin mRNA remaining in oocytes microinjected with either poly(A)-free mRNA alone and with haemin. Increasing amounts of RNA from each sample of oocytes were hybridised to a constant amount of α -globin enriched ^3H -cDNA at concentration where less than 20% of the radioactive probe is annealed at the end of the reaction². In these conditions, the amount of ^3H -cDNA annealed was proportional to the amount of globin mRNA present in the reaction medium for all samples tested (Fig. 2). The slopes of the straight lines corresponding to the four samples of oocytes thus constitute relative measurements of the amounts of globin mRNA present in each batch.

In the presence of haemin only 13% of the injected poly(A)-free α -globin mRNA remained intact at the end of the incubation, whereas 60% of the injected message remained hybridisable to the ^3H -cDNA probe in the absence of haemin (Fig. 2). Similar results were obtained in another experiment using different preparations of α -globin mRNA and ^3H -cDNA as well as oocytes from a different frog.

From previous experiments² and controls data (not presented), it is known that haemin *per se* has no degradative effect on injected native globin mRNA. Thus, the simplest interpretation of our results is that, in *Xenopus* oocytes there is a mechanism for the degradation of poly(A)-free mRNA requiring or strongly activated by translation or at least its initiation.

Puromycin enhances the degradation of a microinjected *Dictyostyleum* RNA preparation enriched in mRNA¹²; this led to the suggestion that the release of mRNA from ribosomes reduces its stability. We cannot explain these results as, using purified native globin mRNA in the same kind of experiments, we did not observe any effect of puromycin on the stability of the message. It should be noted, however, that it has proven impossible to keep oocytes in healthy conditions for long periods of puromycin treatment. One may therefore question the physiological meaning of such experiments. The use of haemin to enhance the translation of α -globin mRNA does not have this disadvantage.

On the other hand, it has recently been claimed^{13,14} that translation, or at least some of the step(s) involved in the process, are required for the degradation of mRNA in different systems. The observations presented here strengthen these claims.

Fig. 1 Relative synthesis of α - and β -rabbit globin chains in oocytes injected with native globin mRNA 5 ng per 50 nl. ●, In the absence of haemin; ○, in the presence of haemin (1.5 mM in the injection medium). Following injection, each set of oocytes was incubated for 15 h at 20 °C in Barth's medium containing ^3H -histidine (50 Ci mol⁻¹, 200 $\mu\text{Ci ml}^{-1}$). After incubation, the oocytes were homogenised with carrier rabbit haemoglobin and globin was prepared from the 10,000g supernatant of the homogenate¹⁵. Globin chains were separated on carboxymethyl-cellulose columns by a pyridine-formic acid gradient¹⁵ and the radioactivity of the eluted fractions was determined in a scintillation spectrometer.

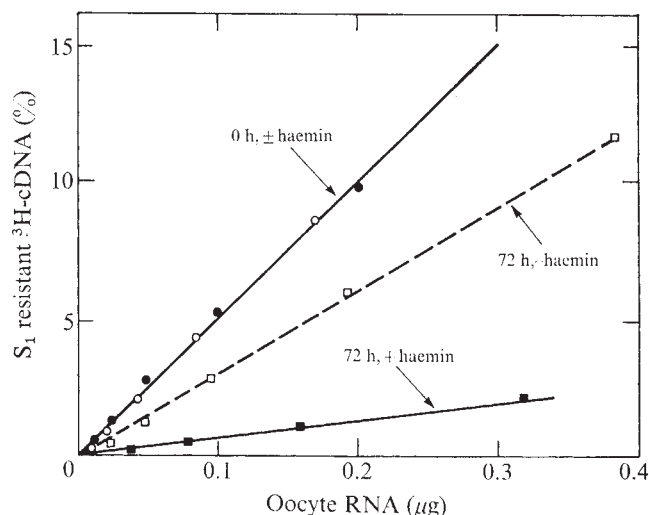
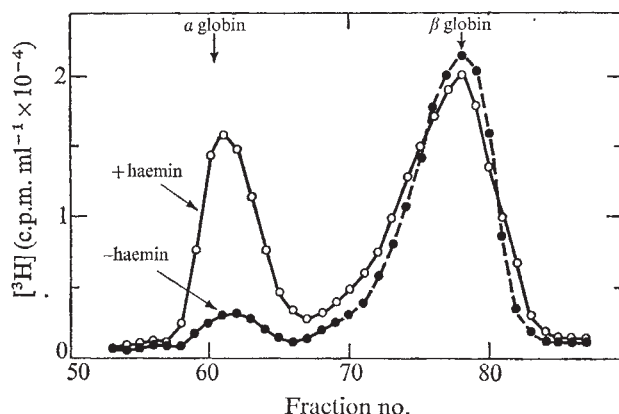


Fig. 2 Effect of haemin on the stability of poly(A)-free α -globin mRNA in *X. laevis* oocytes. Two hundred *Xenopus* oocytes were injected with poly(A)-free α -enriched globin mRNA 5 ng per 50 nl in aqueous solution; 100 oocytes were injected with mRNA in the absence of haemin, and a further 100 were injected with mRNA dissolved in a 1.5 mM haemin solution. Forty oocytes from each batch were frozen immediately after microinjection; the remaining 60 were incubated in Barth's medium at 20 °C for 72 h. Total RNA from each sample of oocytes was extracted using a phenol-chloroform procedure². Increasing amounts of RNA from each sample of oocytes were hybridised to a fixed amount of globin ^3H -cDNA in conditions where the amount of annealed cDNA is proportional to the amount of globin mRNA present in the reaction medium. Hybrids were detected by their S_1 nuclease resistance². Hybridisation of RNA to (^3H) globin cDNA: □, ○, RNA was extracted from oocytes micro-injected with poly(A)-free α -enriched globin mRNA without haemin; □, ○, RNA extracted immediately after injection; □, ●, RNA extracted after 72 h of incubation of oocytes. ■, ●, the same with haemin.

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matters arising

Backwarming due to circumstellar shells

IN a recent publication, Donnison and Williams¹ discussed the possible effects which a circumstellar dust shell could have on the evolution of a premain sequence star surrounded by it. If λ is the fraction of energy emitted by the star that returns back to it, then they concluded that $(1-\lambda)/\lambda$ can have a value as low as

$$(1-\lambda)/\lambda \simeq (T_0/T_1)^4 \quad (1)$$

where T_1 and T_0 represent the temperatures of the inner and the outer surfaces of the shell. We intend here to draw attention to the fact that equation (1) could be misleading since it represents the smallest value that can ever be allowed by the ratio, and is by no means a realistic limit. The full expression for this ratio can simply be derived as follows.

If we assume that the shell stores no energy, then

$$R^2 T_{\text{eff}}^4 (1-\lambda) = \alpha_1 R_o^2 T_o^4 \quad (2)$$

where R and R_o are the stellar radius and outer radius of the shell and T_{eff} is the effective temperature of the star, α_1 is a constant, which is included because the outer surface in reality will not be a perfect black body characterised by the temperature T_o .

Simple geometry implies that the fraction of the radiation emitted by the inner surface which strikes the star is

$$\alpha_2 \mu R_1^2 T_1^4 \quad (3)$$

where α_2 compensates for the inner surface not being a perfect black body and μ is the fraction of the total solid angle which is subtended by the star at the inner surface of the shell, so that $\mu = \beta(R^2/R_1^2)$ with β a numerical constant which is easy to determine if the star is small but more complicated if the star and shell have similar radii. Hence, from (3)

$$\lambda R^2 T_{\text{eff}}^4 = \alpha_2 \beta R^2 T_1^4 \quad (4)$$

From (2) and (4)

$$\frac{(1-\lambda)}{\lambda} = \left(\frac{\alpha_1 R_o^2}{\alpha_2 \beta R^2} \right) \left(\frac{T_o}{T_1} \right)^4 \quad (5)$$

And it is immediately evident that equation (1) as used in the reference¹ is only

applicable to a very restricted set of physical parameters where

$$\alpha_1 R_o^2 = \alpha_2 \beta R^2 \quad (6)$$

In general $R_o \gg R$ and the ratio $(1-\lambda)/\lambda$ is very much greater than the value given by equation (1) (that is, λ is smaller).

We conclude, therefore, that even though it is theoretically possible to have a situation where λ is close to unity, which results in a drastic change in the evolution of the central star, generally, in physical meaningful situations $\lambda \ll 1$ and backwarming has no real effect.

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Possible solar control of North Atlantic oceanic climate

TRENDS in the climate of the North Atlantic Ocean over the past century have been described recently by Colebrook¹. He discussed the variation in the average sea level, the number of cyclones and the sea-surface temperature. He showed that low sea-surface temperatures in the North Atlantic are associated with a higher-than-normal flow in the Gulf Stream and a lower-than-normal frequency of tropical cyclones, indicative of above average intensity in mid-latitude westerlies. The sea-surface temperature data were smoothed by the application of eigenvector filters which were approximately equivalent to 7-yr running means. A 10–11-yr periodicity, which tended to be in phase with the sunspot cycle, was found following the removal of long-term trends from the smoothed data. I point out here a very significant long-term correlation between the sea-surface temperature and the 7-yr running mean of the annual sunspot numbers. I have redrawn the sea-surface temperature data of Colebrook¹ and compared it with the 7-yr running mean of the annual sunspot number for Marsden squares 145 D and 182 B (Fig. 1). In both cases the vertical

scales were adjusted so as to make the total amplitude of the long-term variation approximately the same on the graph.

There is very good correlation between the two sets of data for Marsden square 145 D (45°–50°N,

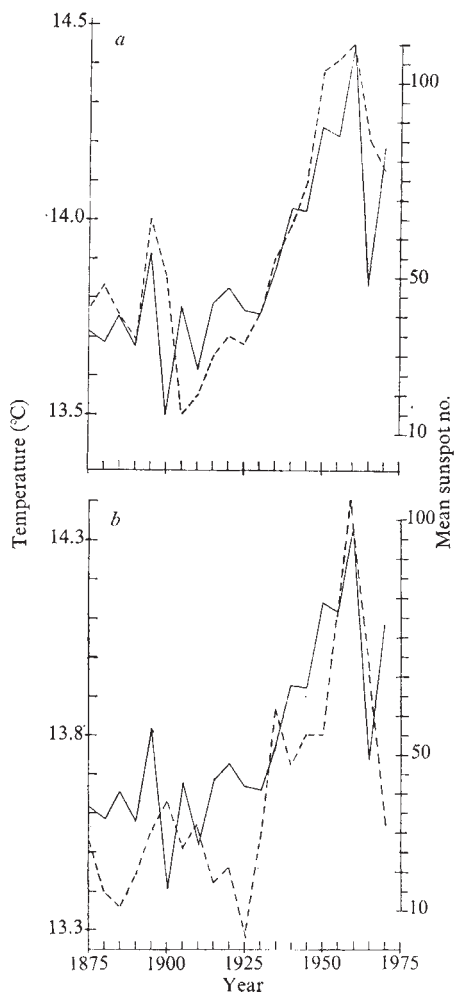


Fig. 1 Smoothed variations of sea-surface temperature redrawn from ref. 1 (broken lines) compared with the 7-yr running mean of the annual sunspot number (solid line). *a* Refers to Marsden square 145 D (45°–50°N, 5°–10°W) and *b* refers to Marsden square 182 B (50°–55°N, 15°–20°W).

5°–10°W) while the correlation is less marked for Marsden square 182 B (50°–55°N, 15°–20°W). This difference in the degree of correlation between the two sets of data might be accounted for by the difference in latitude between the two areas under consideration. The effect of latitude on the

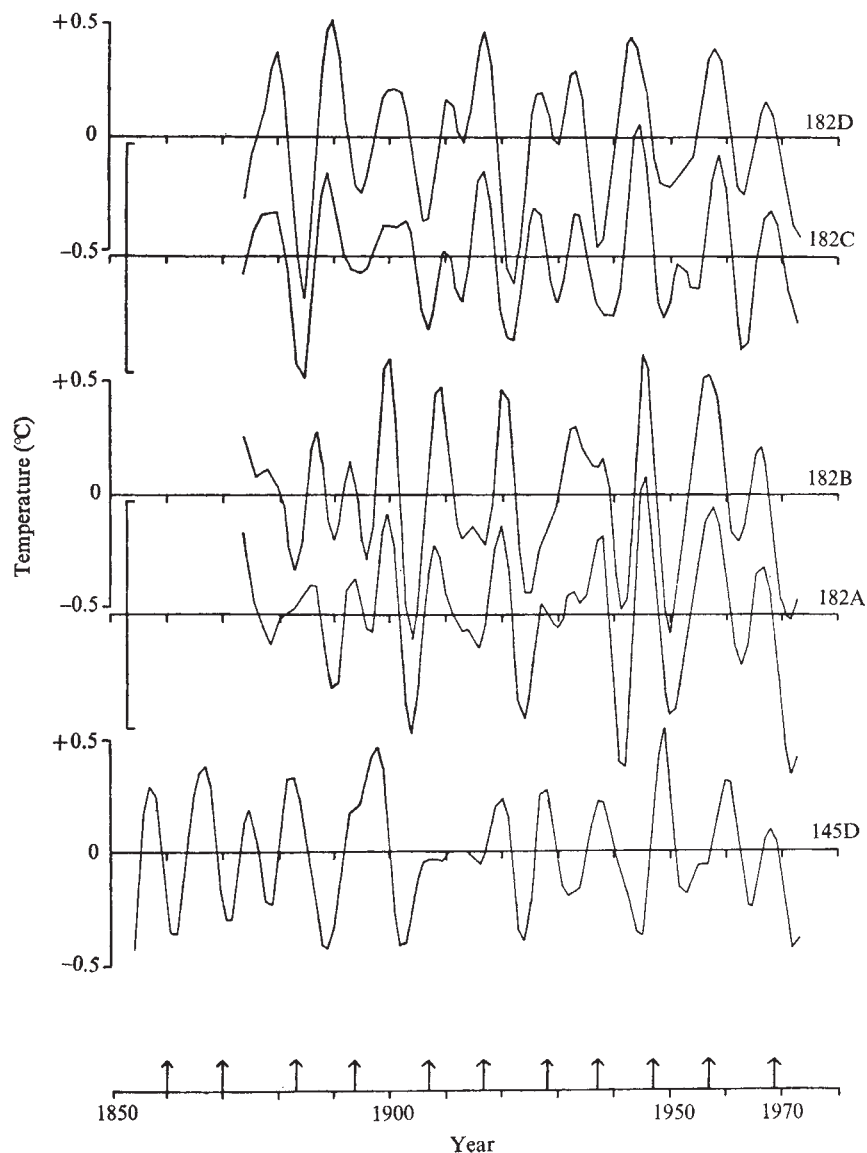


Fig. 1 Filtered mean sea-surface temperatures for quarter Marsden squares 145 (Celtic Sea and Bay of Biscay) and 182 (Rockall area). The variables show the dominant periodic element following the removal of long term trends. Arrows on abscissa indicate years of maximum sunspot numbers.

possible relationship between solar activity and the circulation of the lower atmosphere has been discussed at some length by King².

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COLEBROOK REPLIES—In the light of the very impressive correlation between sea-surface temperature and annual sunspot numbers demonstrated by Muir¹, I comment here in greater detail than was possible before² on the periodicities in sea-surface temperature data for Marsden squares 182 and 145. Figure 1 shows plots of filtered sea-surface temperatures representing the dominant periodic elements in the

series following the removal of the long term trend. In each case there is a marked 10–11-yr periodicity. There is a fairly good phase relationship between the various temperature series and with the sunspot maxima.

It should also be noted that Cohen and Sweetser³ detected an 11-yr periodicity in their series of frequencies of tropical cyclones in the Atlantic.

In the light of all the evidence now available there seems little doubt that sunspot numbers do have an influence on the climate of the North Atlantic Ocean.

I suggested previously² that the effect on sea-surface temperatures in the north-east Atlantic was produced by variations in southerly anomaly winds, involving direct heat exchange with the atmosphere. The relationship found by Muir implies an influence on the atmospheric circulation over the whole North Atlantic with a consequent

effect on the Gulf Stream. This is consistent with the results presented by Maximov⁴ which indicated that the 10–11-yr cycle in sea-surface temperature occurs over most of the North Atlantic Ocean.

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Oxygen isotope ratios in spruce cellulose

ALTHOUGH not affecting the practical significance of their work, Gray and Thompson's¹ finding that oxygen isotope fractionation in the cellulose of white spruce (*Picea glauca*) depends on winter rather than summer temperature warrants further discussion. It is unlikely that the tree is photosynthesising to any significant degree in the winter. I suggest here an alternative mechanism which would produce the observed results.

Two facts argue against winter photosynthesis: First, Fritts's² conifer, cited by Gray and Thompson, photosynthesised only when the temperature was above freezing and soil water stress was low—two conditions unlikely to hold for long periods in the Edmonton winter. Second, Norway spruce (*Picea abies*) chloroplasts function in carbohydrate synthesis only during the summer months³. If this is also true for white spruce, winter photosynthesis is probably not possible.

For energetic reasons a heavier isotope accumulates preferentially in that molecule having the highest bond multiplicity at the point of isotopic substitution^{4,5}. Thus, when ¹⁶O and ¹⁸O distribute themselves between H₂O and CO₂, the CO₂ (in which oxygen is double bonded) will get proportionately more ¹⁸O than the H₂O (in which oxygen is single bonded). This effect decreases with increasing temperature⁶. Therefore, if one looks at oxygen from CO₂ which has been in solution with H₂O, the δ ¹⁸O-temperature curve will have a negative slope. But, Gray and Thompson's curve has a positive slope. The oxygen in their cellulose might have come from H₂O rather than CO₂, but this is not consistent with our current understanding of photosynthesis. Alternatively, starch may be hydrolysed to glucose within the cell during the winter. The glucose (single-bonded oxygen) would then exchange oxygen with cellular organic acids (or any other molecules containing double or

triple oxygen bonds), producing the observed $\delta^{18}\text{O}$ -temperature curve.

The behaviour of Norway spruce chloroplasts³ seems to support this idea. In autumn, they function primarily in starch storage. In winter, they group together and membranes become disorganised. In spring, they again store starch—of necessity the same starch which was stored in the autumn. This mechanism suggests that the bulk of radial growth in the tree is produced by the previous year's photosynthate, which is contrary to accepted thinking⁷.

Hydrolysis of starch to sugars in the winter could have functional value. Sugar solutions protect at least some cell functions during freezing⁸. Heat of hydrolysis could conceivably provide a significant warming effect within the cell.

I thank Dr James Pickett for helpful discussions.

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Isotope tree thermometers

WE wish to make several comments on the recent paper in *Nature* by Libby *et al.*¹

They determine the $\delta^{18}\text{O}$ in wood by analysing the CO_2 produced by heating the total wood samples with HgCl_2 .

$$\delta\text{D}_{\text{‰}} = \left(\frac{\text{D/H sample}}{\text{D/H standard}} - 1 \right) 1000$$

The standard is usually standard mean ocean water (SMOW). $\delta^{18}\text{O}$ is defined similarly. The standard used in Libby *et al.*¹ is as given in their text. Neither Libby *et al.*¹ nor the original developers of this method² have shown that this procedure is valid for the high accuracy necessary in this work.

The δD data shown in their Fig. 3 (ref. 1) was originally published elsewhere³ where they reported that these δD analyses were made on H_2 released by pyrolysis of a mixture of wood sawdust and powdered uranium. In their *Nature* article¹ they state that this same δD data were obtained by combustion of the wood with oxygen and passage of the resulting water over hot uranium. This apparent contradiction

should be cleared up, since only the latter method has been shown to be reliable^{4–6}.

They assume that the $\delta^{18}\text{O}$ and δD values of the total wood can measure by proxy the $\delta^{18}\text{O}$ and δD of meteoric water used by the plants. It is the isotopic composition of the meteoric water which is the 'thermometer'. Their assumption is incorrect⁶. It is known^{5,6} that the δD of lipids in the wood can be 100% lower than the δD of the total plant. Thus even if correct δD analyses are made on total wood, this δD also depends on the wood's variable chemical composition which is not temperature sensitive⁶.

Their sole criteria for a valid relationship between temperature and the δD and $\delta^{18}\text{O}$ values of total wood is the existence of doubtful correlations between the isotope records in dated tree rings and the time-equivalent instrumentally recorded temperatures in cities. Their choice of climatic data for correlation purposes is subjective. For example, the isotopic data of their Fig. 1 is correlated with average annual temperature, whereas, in their Fig. 3 the correlation is between isotopic data and English winter temperature. Their data bear little correlation with the climatic history of Europe as estimated by Lamb⁷.

Some of their data are very unusual. The δD for the German oak tree rings shown in their Fig. 3 changes by 150‰ between 1820 and 1940 θ^- and 190‰ between 1720 and 1820 θ^- . This change is about three times as large as the δD change between summer and winter precipitation in Vienna in their Fig. 4, which corresponds to a 20 °C change in temperature. Also there is only a 180‰ range in the δD of average precipitation available to plants growing from tropical to subarctic temperatures⁶ (Fig. 1 below). These changes in δD values in the oak tree could not be due to changes in δD of the precipitation in Germany nor to climatic changes. Similar questions can be raised about the unusually large changes in $\delta^{18}\text{O}$ in their Fig. 1¹.

We have made some $\delta^{18}\text{O}$ measurements on cellulose from tree wood and other plants (S. G., P. Thompson and C. J. Y., unpublished) and found that the relationship between the δD and $\delta^{18}\text{O}$ in cellulose is complicated by factors such as evaporation–transpiration in the plants. There is no *a priori* reason to expect the δD against $\delta^{18}\text{O}$ relationship to be similar to those for meteoric waters. Thus their claimed slope of 8 for the relationship between the $\delta^{18}\text{O}$ and δD for total wood shown in their Fig. 6, and the conclusions they draw from this figure are puzzling. The slope 8 is not by itself an indication that the tree is storing old water, nor does it support their stated hypothesis that atmospheric CO_2 and atmospheric H_2O are in isotopic equilibrium. The $\delta^{18}\text{O}$ of the atmospheric CO_2 varies little and is basically determined by the equilibration of CO_2 with ocean water^{8–11}.

Fourier analyses of a continuous 2,000-yr record of δD and $\delta^{18}\text{O}$ analyses for five-year intervals of tree rings should reveal the presence of shorter cycles than reported by them. It is difficult to understand why the shorter cycles were not included in their Fig. 7 considering their expressed interest in finding a 21-year cycle.

We conclude that the δD and $\delta^{18}\text{O}$ as determined by Libby, *et al.* on total wood do not give information about climate. On the other hand, we have shown that the δD analysis of the hydrogen from only the C–H groups in cellulose (isolated as nitrated cellulose) eliminates the problems of chemical heterogeneity and the exchange with water of the hydrogen in the

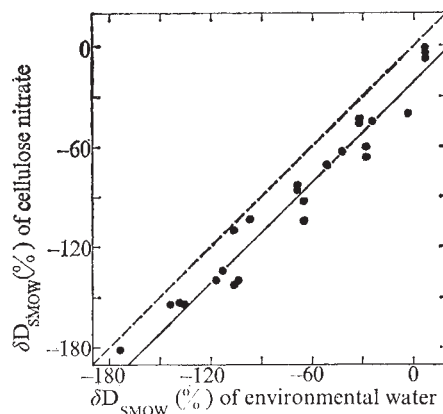


Fig. 1 The plot of δD of plant-derived cellulose C–H hydrogen (cellulose nitrate) against δD of environmental waters used in the plants yields a linear regression curve (solid line) with a slope of 0.97 and intercept of -22‰ . The linear correlation coefficient is 0.978. The dashed line is the $y = x$ curve. The samples include varieties of both land and aquatic plants from a wide geographic range.

OH groups⁶. The δD values of the cellulose C–H hydrogen and of the associated OH groups⁶. The δD values of the cellulose C–H hydrogen and of the associated environmental water were compared for 20 different species of land and aquatic plants including nine different species of trees, collected from areas including Scotland, the Yukon Territory, Wisconsin, California, Texas, and Miami, Florida. The comparison is shown in Fig. 1. The δD of the C–H hydrogen is, on the average $-20 \pm 11\text{‰}$ (σ) relative to the δD of the environmental water. Thus, the δD of cellulose C–H hydrogen from plants systematically reflects the δD of the H_2O incorporated by them and should be a good climatic thermometer.

Historically, Libby *et al.* should note that, by 1970, at least two laboratories^{4,5} were measuring the isotopic composition of plants in order to determine by proxy the isotopic composition of meteoric water.

As far as the item on future research is concerned, we have already done much of what Libby *et al.*¹ recommend,

including the identification of a 22-yr cycle in a 1,000-yr δD record¹² in the bristlecone pine of the White Mountains, California. We also analysed forty trees ranging in age from 9,000–22,000 yr to obtain a most interesting record of δD of meteoric water during the Wisconsin glacial period¹³.

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LEONA MARSHALL LIBBY AND LOUIS J. PANDOLFI REPLY—Long-term isotope changes in precipitation, caused by changes in climatic temperature, are well documented in polar ice caps; the heavier of the stable isotopes is depleted in ice laid down in the ice age by comparison with present-day ice. In 1970 we extended this concept to trees, suggesting that they are also thermometers. In trees which use rain to manufacture their rings, isotope variations in the rings should be climate indicators because the isotope composition in rain and CO_2 varies with temperature.

On May 17 1971, the Advanced Research Projects Agency funded our proposal that "temperature variations may be evaluated by measuring stable isotope ratios in natural data banks such as tree rings and varves." L.M.L. had previously calculated¹ the theoretical temperature coefficient of the isotope fractionation in manufacture of wood from CO_2 and H_2O , finding that the coefficient is small compared with that measured in rain.² Therefore isotope variations in rain should be dominant in trees that drink rain.

The next question is what to measure: whole wood, cellulose, lignin . . . ? It may be true that the ratio of cellulose to lignin differs in spring and summer wood and over spans of decades.³ Tentatively it seems that there is more lignin when it is warmer as in later summer, and that lignin is more enriched in the heavy isotope. We choose to regard such

variations as part of the response of trees to climate variations, namely as part of the record of climate history.

Thus it is valid to measure any one of these substances, depending how faithfully it tracks local mercury thermometers. Our measurements on whole wood track for both hydrogen and oxygen. We expect the same success for cellulose and lignin, but they will track with different amplitudes because in these compounds different sets of chemical bonds are fractionating when the temperature changes.

In the development of radiocarbon dating the same question arose whether to measure wood, cellulose, or lignin, that is the $^{13}C/^{12}C$ and $^{14}C/^{12}C$ ratios will be different in each of these materials just as D/H is. W. F. L. chose to measure whole wood.

The next problem is what chemistry to use. There is compelling evidence that when sapwood passes into heartwood it becomes sealed against exchange with sap.⁴ It is also known that isotope exchange occurs when wood is finely ground and soaked in hydrogen containing liquids⁵. So we decided to use dry chemistries. For our first tree sequence^{6,7} we measured D/H by reacting sawdust with uranium to release H_2 , 99% quantitatively. For the later tree sequences⁴ we burned wood to water and reacted it with uranium to release H_2 . For measurement of $^{18}O/^{16}O$ we modified the method of Rittenberg and Pontecorvo⁸ by carrying it out at very high temperatures, 99% quantitatively.

Our third problem was which trees to study. We could not use bristlecones because there are no lengthy temperature records for hundreds of miles near where they grow and we were determined to calibrate the trees against local mercury thermometers. Because the longest temperature records are in Europe, we obtained a German oak from the only tree laboratory then existing in Europe⁴.

For measurement of the oaks, we used per force a mass spectrometer of somewhat low accuracy, and achieved the accuracy necessary to demonstrate tree thermometers by making many measurements on each sample. On the later tree sequences⁴ we used high-precision machines with accuracies of ± 0.1 p.p.t. for $^{18}O/^{16}O$ and ± 2 p.p.t. for D/H. Whether the oxygen in tree rings comes from water or from CO_2 is a non-question, because Cohn and Urey⁹ showed that isotopic equilibrium between the two substances is obtained in the atmosphere in a few hours.

The least square correlation of D/H against $^{18}O/^{16}O$ in the 1,800 yr sequence yielded the relation,

$$\delta D = 8.2 \delta^{18}O + \text{constant}$$

which is the same as in worldwide rain¹⁰ within experimental error. If both the hydrogen and the oxygen in the OH

groups exchanged with water vapour there is absolutely no way that the slope can be 8, because there are twice as many hydrogens bonded to carbon as to oxygen.

The Fourier transforms⁴ of our five-year samples in the 1,800 yr sequence showed ten significant periods ranging between 50 and 300 yr, the same for D/H and for $^{18}O/^{16}O$. To look for periods of less than 50 yr in a sequence of five-year samples or to look for periods of more than 300 years in a sequence of 1,800 yr is meaningless. Such periods appear in the transforms but they do not necessarily represent reality. To understand this we generated random signals of values 0 and 1, and assigned them to 10-yr intervals of a 1,000-yr hypothetical sequence, and made a Fourier transform. In three such computer experiments we always obtained periods at 10 and about 20yr which are artefacts of the sampling interval. These statements can be understood by reading carefully ref. 11, particularly pages 30–33.

Obviously ring samples integrated over 5 or 10 yr cannot give proof of the 21-yr solar cycle; instead each ring must be measured.

Nine of our ten periods⁴ are equal to¹² those evidenced in the Greenland ice wells.¹³ One of the periods, that at 173 yr, has also been found by Houtermans and Suess¹⁴ in bristlecone pines.

Our isotope ratios track better with winter temperatures than with the average temperatures. Dr John Fletcher suggested that when climate deteriorates summer is brief and January, February, and March represent the longer winters.

For the cellulose component of wood, Epstein and Yapp¹⁵ and Gray and Thompson¹⁶ have corroborated our work.

Tree-ring thermometry promises to evaluate past climates and predict future climates.

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reviews

Interferon as an anti-viral

Thomas C. Merigan

Interferon and Its Clinical Potential. By D. A. J. Tyrrell. Pp. viii+104. (Heinemann Medical: London, 1976.) £3.50.

THIS brief and timely monograph clearly presents the author's personal views of the interferon area. The 91-page review covers two decades of intensive research which has formed the basis for the present clinical investigations involving interferon, following its discovery in 1957. This remarkable molecule is not only of clinical interest as an anti-viral, but there is an emerging body of information supporting its role as a locally active cell regulator with a broad range of functions beyond its anti-viral action. It not only seems to influence malignant, immune and haematopoietic cell division, but certain specialised cell biosynthetic functions as well. This viral inhibitor discovered by Alick Isaacs now also attracts the efforts of molecular biologists as cell-free systems have been developed in which it can be produced and in which anti-viral action can be demonstrated. The recent discovery of cell membrane receptors for interferon, which seem to be gangliosides, should now allow elucidation of the mechanism of the first step in its action—that is, its binding to cells.

The book provides the clinician, basic virologist, and specialised investigator with a firm basis for understanding the potential of interferon in human medicine. It is digestible in an evening of solid reading by anyone with an understanding of modern biology. Although clinical trial results are appearing with greater frequency and views on its mechanism of action will be sharpened as more is learned of the fundamentals of viral replication, the basic description of the interferon system presented in this volume will be useful to future investigators.

The enthusiasm of the writer and his clear style of writing leads the reader through the voluminous recent literature in this area. One hundred and sixty-five carefully selected references are used to support a very readable discussion of interferon action and production in cell culture, animal models and human disease settings. Present concepts of the assay, properties and purification of interferon are also dis-

cussed. A number of well cast tables and easy-to-comprehend diagrams aid the reader in following the text. The methods for preparation and purification of human leukocyte interferon for clinical use, developed in Finland and in the UK, are discussed by the author, who was chairman of the UK Scientific Committee on Interferon, a group which planned and supported much of the early work with the material. The

long experience of the author in this area insures the reader that the book accurately presents the basic biology of the interferon system, together with present accomplishments and perspectives for its use as a clinical anti-viral and anti-tumour agent. □

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Arroyos in the American South-West

Arroyos and Environmental Change in the American South-West. By R. U. Cooke and R. W. Reeves. Pp. xii+213. (Clarendon: Oxford, 1976.) £8.50.

LARGE gullies with near vertical walls and flat, sandy bottoms, dissecting the alluvial valley floors of the American South-West, have attracted the attention of geologists and geographers for several decades. The arroyos, as they are called, seem to have developed after 1850, about the time of white settlement and the introduction of livestock in large numbers to the western plains. Not unnaturally, it was commonly supposed that land-use changes were responsible for the erosion, on account of depletion of the vegetation and greater runoff. Sedimentary sections showed, however, that there had been earlier periods of trenching in prehistoric times, and it was suggested that slight climatic changes were at least partly responsible.

It is known that overstocking occurred in Arizona about the same time as severe drought in the 1880s, and it is clear from J. R. Hastings and R. M. Turner in *The Changing Mile* (University of Arizona, Tucson, 1965) and from other sources that the vegetation has deteriorated markedly since that time. Cooke and Reeves show that in the coastal regions of southern California where arroyos also occur, rainfall records provide no support for the idea that secular or cyclic changes in precipitation have played a determining role in the formation of the arroyos. Furthermore, they present evidence that calls into question much that is commonly accepted about the effects

of land-use on runoff coefficients.

Using archival records of various kinds, and in particular the field notebooks of the US General Land Office surveyors, Cooke and Reeves trace the development of a large number of individual arroyos. As they acknowledge, the extension of certain Californian arroyos was documented by W. B. Bull (*Am. J. Sci.* **262**, 249–258; 1964). Their own study is much more extensive. It demonstrates that wherever the history of an arroyo can be traced, individual events and acts of interference by man have been responsible for its initiation and enlargement. Cultivation of valley floors and destruction of vegetation along shallow watercourses have usually played an important part; but in the case of the San Simon arroyo, for example, a wagon route seems to have been the immediate cause of gullying, whereas the Santa Cruz arroyo was initiated by water supply channels. Elsewhere, concentration of drainage water by culverts or other man-made features started the downcutting.

Arroyos have destroyed floodplains, accentuated flood peaks and accelerated sedimentation to the detriment of the environment of the American South-West. We now know that they are the outcome of a multitude of individual events and that sweeping explanations are inadequate. Cooke and Reeves have labouriously but effectively sawn through the Gordian knot tied by earlier investigations.

A. T. Grove

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Stereochemistry

Stereochemistry: An Introductory Programme with Models. By J. Pearce and E. Glynn. Pp. 171+cut-out kit. (Wiley: London and New York, 1976.) £2.75; \$5.

TEACHERS involved in imparting the basic principles of stereochemistry in organic molecules to their students are always faced with the problem of persuading the students to purchase sets of molecular models and to use them to build three-dimensional structures, thereby gaining a better understanding of stereochemistry. Pearce and Glynn have produced a kit of models and shapes together with a programmed text containing clear and detailed instructions, so that a student may work at his own pace, in discovering the essential principles of stereochemistry and conformation. A number of fascinating shapes are provided which enable the student to gain an understanding and appreciation of chirality and its importance in stereospecific reactions in both chemical and biochemical systems.

The models are of the well-known 'orbit' type and are easily assembled; cyclohexane and its derivatives in particular are easily constructed and manipulated. The programmed text includes a progressive series of questions for the student to answer, often as a result of the assembly and study of models or shapes. The answer is provided after each ques-

tion and at the end of each section the essential principles relating to it are reviewed.

The text begins with a discussion of the various types of molecular models, and instructions relating to the construction of models of simple covalent molecules using the kit provided. Conformations of open-chain and ring compounds are discussed, followed by a consideration of chirality and restricted rotation. The various strategies for examining molecular shapes are explained, and the objectives of the learning program summarised. A series of appendices are provided at the end of the text, some of which the reviewer would have found more useful if given either at appropriate places in the text or at the beginning. The text might also have benefited from a more detailed consideration of the D/L and R/S conventions.

This is an excellent programmed text and set of models and shapes for a student to use in discovering the principles of stereochemistry. It is good value for money and can be strongly recommended. A larger set of models attached to the text, however, would be desirable for long-term use to enable the student to build larger models, pertaining to his own interests and possibly outside the range of the text.

D. I. Davies

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Aspects of hydrology

Facets of Hydrology. Edited by John Rodda. Pp. xvi+368. (Wiley: London and New York, 1976.) \$39; £19.50.

THIS book is a bold attempt to bring together, in a single volume, papers describing much of the hydrological work which has been undertaken in the past decade. Most of the authors are internationally recognised experts, although a few are little known outside their own country.

The stated aim of the book is to provoke the interest of a range of readers, both researchers and practitioners, who are hydrologists or scientists working in allied fields. These aims are achieved with a fair measure of success but, as is often the case with a book produced for a wide and diverse audience, it is not entirely successful. At the one extreme some chapters may be regarded as superficial by experts in the particular subject; at the other there are chapters which are written for readers who themselves already have a very specialised knowledge.

Nevertheless, every reader will find much of interest in a book which will enable him to acquire knowledge of many of the facets of hydrology with which he was not familiar.

It is not possible to comment on each of the chapters in the book and to single out any particular chapter is simply to reflect the reviewer's own interests. The chapters on river gauging, water quality, basin studies, statistical methods, and law, however, each demonstrate in their own way, the diversity of material presented.

In the chapter on river gauging three new methods are discussed with a sensible balance between theory and practical applications. The fact that there is a chapter on water quality will be particularly welcomed by those people who have always argued that the division between water quality and water quantity is an artificial one. The chapter on basin studies presents a representative selection of the very wide range of work undertaken in recent years. That on statistical methods is one of the chapters requiring a fair degree of specialist knowledge. It tackles the problem of spatial variation in hydrological variables in a way which will be extremely useful to those people who are confronted with the problem. The chapter on law is a comprehensive review of the water law and will be of interest to all water resource engineers.

The overall impression is of a book which should find its way on to the shelves of most serious hydrologists and of all well stocked libraries.

M. J. Hamlin

M. J. Hamlin is Professor of Water Engineering at the University of Birmingham, UK.

Kew Record of Taxonomic Literature

This comprehensive annual publication indexes literature relating to the vascular plants of the world and includes all new names at all ranks with the exception of cultivars. No other single publication fulfils this need for unified coverage of the world's taxonomic and related literature. Since 1974 four volumes have been published, and volumes for literature published in 1975 and 1976 are at press.

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Zeolite catalysis

Zeolite Chemistry and Catalysis. (ACS Monograph, 171.) Edited by J. A. Rabo. Pp. viii+796. (American Chemical Society: Washington DC, 1976.) \$65.

In the preface to D. W. Breck's *Zeolite Molecular Sieves* (Wiley-Interscience, 1974), the author states that the important subject of zeolite catalysis was deliberately omitted. This serious omission has now been rectified by the publication of the only other major work in this field, edited by Jule Rabo, an associate of Dr Breck at the Union Carbide Corporation. All zeolite chemists now have two texts to keep on hand, which between them cover all the aspects of the subject and survey many important papers published up to the end of 1973.

It is a pity, however, that a book as costly as this one could not have been published more quickly, so that there

is not such a wide gap in the literature. The book has, however, been carefully edited. There are only a small number of printing errors, most of which are obvious to a reasonably well-informed reader.

Six of the chapters are written by personnel from the Union Carbide Corporation, making the book somewhat biased in its outlook. This is a pity considering that much important work in this field is being carried out in European and Japanese laboratories. It is also a pity that a chapter emanating from the Mobil laboratories, where some of the most important research in this field has been carried out, could not have been included.

Despite these criticisms, this is an excellent book which will be an essential acquisition for all those working in this field.

L. V. C. Rees

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Unity of chemistry

Inorganic Aspects of Biological and Organic Chemistry. By R. P. Hanzlik. Pp. xvii+402. (Academic: New York and London, 1976.) \$37; £26.25.

ALTHOUGH I agree whole-heartedly with the intentions expressed in the foreword to this book I am somewhat dissatisfied with the way in which they have materialised. It is true that interdisciplinary areas such as those between inorganic and organic chemistry (organometallic chemistry) and between inorganic and biochemistry (bioinorganic chemistry) provide a teaching opportunity which, if properly accepted, could show a student the unity of chemistry. The obvious danger is that, while stressing the interdisciplinary elements of the topic, the clear strengths of the disciplines themselves are lost.

The title tells us that the three disciplines we need are those of Inorganic Chemistry, Organic Chemistry and Biology. The author tackles inorganic chemistry bravely in the introductory chapter and covers the organic chemistry well in later chapters (that is, its inorganic aspects). As there are many good texts on organometallic chemistry, however, I looked for something extra which would provide the essential link with biology. Surely this must be through topics such as oxygen insertion, Grignard reagents, hydrogen activation, ester and amide synthesis, hydrolysis, and so on.

I feel then that I should have been given detailed contrasts between peroxidase, cytochrome P-450 and Fenton's reagent; magnesium Grignard reagents and vitamin B₁₂; Wilkinson catalysts and hydrogenases; and between Lewis acid-base catalysts and metallo-enzymes. Unfortunately many of these topics receive scant treatment. The reason is I believe a lack of insight into biology, and I therefore look upon this volume as a brave and worthy attempt, rather than a great success, for I miss the special features of the discipline which is biology.

Surely the staggering feature of all biological chemistry is organisation without simple order. Even trivial compounds such as calcium carbonate have to be regarded with different eyes when they appear before us in shells, with their enormous variety of shape and form. Again, to mention cytochromes and not the organised system of catalysts of photosynthesis, or to leave out chlorophyll and its special organised function in the leaf, or to omit halogenation by peroxidases and the relation to immune systems, leaves the feeling that the inorganic aspects of biological chemistry are not seen in their true depth.

Dr Hanzlik has produced a good book, but there was the material here for a really wonderful one.

R. J. P. Williams

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Insect viruses

Virus-Insect Relationships. By Kenneth M. Smith. Pp. viii+291. (Longman: London and New York, 1976.) £11.

A NEW BOOK on insect viruses is a rare and welcome event for those who work in this area, so a first reaction to it is naturally one of enthusiastic interest. This book is a well produced volume, nicely laid out and printed in a clear easily read type. There are many illustrations but most of the electron micrographs are marred by their flat grey tones. It is a pity that closer examination soon tempers this first impression. Some fears are aroused by certain comments in the preface: "This book has been written by one of the few remaining old-fashioned virologists" and "detailed accounts of the chemical nature of the viruses and their molecular biology have been omitted". Is this an apology for an incomplete or outdated treatise?

The book is divided into two parts, one giving an account of the types of virus and virus disease affecting insects, the other discussing more general aspects such as infection and replication. Although there are advantages to such a layout, it does mean that repetition occurs and narrative lacks cohesion. The further division of the chapters in the first part of the book into a general account of the virus group followed by descriptions of specific viruses was ill-advised. Both repetition and contradiction occur as a result. The general account component of each chapter decreases progressively from two and a half pages in chapter 1 to four sentences in chapter 7 so possibly the author by then had appreciated the difficulties that this approach creates. The book has other structural peculiarities. Just one example is the grouping together of parvoviruses and picornaviruses in one chapter (with a short general introductory account dealing with parvoviruses only), whereas viruses of the honey bee and *Drosophila* species are deemed worthy of a separate chapter despite the fact that most of them are small spherical RNA-containing viruses anyway.

The chapters in this first part of the book describe the various groups of insect viruses mainly from a morphological standpoint, with only spasmodic attempts to include relevant biophysical or biochemical data. More recent efforts in insect virus research, such as the definition of methods and criteria for identifying the large number of virus isolates from different insects more reliably than by naming the host insect, are given scant attention. Not only are such recent data omitted but

certain accounts also seem outdated conceptually and there are several examples quoted of observations or hypotheses which would not now find great support. There are some errors also. For example, the 1.38% DNA quoted for one insect poxvirus has since been corrected to nearer 5%, and Fig. 5.6 is in fact a picture of *Wiseana cervinata* iridescent virus and not that of *Costelytra zealandica*.

In the second part of the book the chapters on serology, nucleic acids and tissue culture in particular are poorly done. Similarly the account of possible hazards in applying insect viruses as pesticides does not do justice to the amount of time and effort spent on this topic in the last three years. Surprisingly it is only in this chapter that one can find any mention of the epizootiology of insect virus diseases. I am surprised also to find staining methods for optical microscopy included as a separate chapter when little effort has been made to describe laboratory techniques elsewhere. For example, the chapter on insect cell culture gives no details of available cell lines or media (in contrast to the chapter on insect rearing where details of synthetic diets are provided) and the reader is referred to somewhat out-dated sources of information.

Two chapters are included on vectors of viruses. The one on "Insects and other arthropods as vectors of plant viruses" is a useful general account, although many plant virologists will be surprised to read that semi-persistent or

circulative viruses show no latent period in the vector and do not survive after a moult. One confusing aspect of this chapter is that its subdivision by headings of different type face and size seems quite illogical. The chapter on "Insects and other arthropods as vectors of animal viruses" is of little value. It is woefully brief for a topic of this magnitude and importance, and the examples described appear to have been chosen at random. Both of these accounts seem rather out of place in the book, encroaching as they do on other major areas of virology, and they should perhaps have been given a separate section at the end.

The impression left after reading this book is that sadly it is an opportunity missed. It is quite uncritical and often strangely unbalanced in that examples of work have been selected for description, and others excluded, apparently without valid reason. Insect virology has become a more challenging and exciting subject in the last five years. The book conveys little of this to the uninformed reader. To the specialist it is a disappointment. But this book has no competitors and therefore it has some value and usefulness simply because it is a modern text on insect viruses. As such it will find a place on library shelves as a teaching aid and a reference source. We deserved better.

K. A. Harrap

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Iron-sulphur proteins

Iron-Sulfur Proteins. Vol. 3: Structure and Metabolic Mechanisms. Edited by W. Lovenberg. Pp. xiv+443. (Academic: New York and London, 1977.) \$39.50; £28.05.

SINCE the publication of volumes 1 and 2 in this definitive series in 1973 a number of significant advances have been made in the study of iron-sulphur proteins. These are reviewed in this volume which again is a 'must' for anyone interested in the biology and chemistry of iron-sulphur proteins.

Beinert's chapter on the iron-sulphur centres of the mitochondrial electron transfer system covers "those developments of the last five years that have made the cytochromes minority constituents". The multiplicity and diversity of iron-sulphur centres makes it necessary to have an electron paramagnetic resonance apparatus to study electron transport in mitochondria. It is a pity that the iron-sulphur centres in photosynthetic membranes were not dealt with similarly.

The enzymology of nitrogenase has advanced significantly and is excellently reviewed by Orme-Johnson. The genetics of the nitrogen-fixing (*nif*) gene and the regulation of these genes is the subject of Valentine's chapter. The transfer of *nif* genes to non-nitrogen-fixing organisms is an exciting possibility for the future.

Mössbauer spectroscopy has been crucial in the understanding of the electron transfer properties of the iron-sulphur centres. Two chapters by Cammack, Dickson and Johnson, and by Debrunner *et al.* review the evidence which has been used to adduce structures of the 1Fe, 2Fe and 4Fe centres, and help confirm X-ray evidence discussed separately by Carter.

Finally, the chapter by Holm and Ibers on synthetic analogues of the active sites of iron-sulphur proteins describes the status of their work. Holm's group has synthesised analogues of 1Fe, 2Fe and 4Fe centres. The chemical and physical properties of these centres are summarised, and make beautiful reading. D. O. Hall

D. O. Hall is Professor of Biology in the School of Biological Sciences at King's College, University of London, UK.

Recent Scientific and Technical Books

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- CAHEN, M., and FLATO, M. (edited by). *Differential Geometry and Relativity* (A Volume in Honour of Andr  Lichnerowicz on his 60th Birthday.) Pp.xi+304 ISBN-90-277-0745-6. (Dordrecht and Boston Mass.: D. Reidel Publishing Company, 1976.) Dfl. 80; \$29.50.
- Korrigierter Nachdruck der Erste Auflage. (Heidelberger Taschenb cher, Band 179.) Pp.xi+219. (Berlin und New York: Springer-Verlag, 1976.) DM 16.80; \$6.90.
- LEITMANN, George (edited by). *Multicriteria Decision Making and Differential Games*. (Mathematical Concepts and Methods in Science and Engineering.) Pp.xiii+461. ISBN-0-306-30920-3. (New York and London: Plenum Press, 1976.) \$42.
- PETER, R zsa. *Jeux avec l'Infini: Voyage   Travers les Math matiques*. Traduit du Hongrois par Georges Kassai. Pp.305. ISBN-2-02-004568-0. (Paris: Editions du Seuil, 1977.) np.
- PUTTASWAMAIAH, R. M., and DIXON, John D. *Modular Representations of Finite Groups*. (Pure and Applied Mathematics: a Series of Monographs and Textbooks.) Pp.xv+242. ISBN-0-12-568650-1. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$23.50; £16.70.

Astronomy

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obituary

W. H. Morton

WILLIAM H. MORTON, for seven years Assistant Professor of Geology at Addis Ababa University, Ethiopia, was shot to death by Government troops in Addis Ababa on 10 March 1977, one of the many victims of the current political disturbances in Ethiopia.

Bill Morton graduated from Manchester University and served his apprenticeship with the Geological Survey of Uganda. A field geologist *par excellence*, he belonged to that select group of British geologists with detailed first-hand knowledge of the remoter parts of East Africa.

In addition to his own research publications, always the fruit of long months of painstaking geological mapping with his colleagues and students, Bill had a very real desire to share his hard-earned geological knowledge with students and teachers throughout the country. He had recently completed a handbook for non-specialists on the

rocks, minerals and fossils of Ethiopia, and was awaiting the comments of his colleagues. Its publication may have to await happier times. Of more immediate value to the government of Ethiopia are the geological maps of many parts of the country, including the Addis Ababa region, which Bill and his former students produced with that attention to detail and high degree of intellectual honesty which were amongst his outstanding qualities.

The Afar Triangle has stimulated new concepts in geology which have reper- cussed far beyond Africa, and Bill Morton made his own contribution to the knowledge of a region that has attracted so many distinguished earth scientists. Starting with a recognition that the tilted fault-blocks bordering the Afar were the result of extension tectonics and not, as previously thought, of folding and decompressive stresses, Bill and his colleagues went on to develop a model of crustal attenuation as part of a continuous process of plate

separation. His studies of Afar tec- tonics, of the age trends of plateau and rift margin volcanics in Ethiopia, and of crustal thinning at rift margins, some of which appeared as seminal papers in *Nature*, were always con- veyed in clear and simple prose, a by-product of his experience as a teacher.

A level-headed and thoughtful per- son, Bill Morton was a tireless teacher, devoted to his students and immensely concerned with imparting practical and worthwhile knowledge. He was equally at home on the summit of a glaciated volcano as at the bottom of Ethiopia's deepest known pot-hole. Well loved by students and colleagues, he was highly respected by geologists from many countries. In a tragic moment, Ethio- pia has lost a man who had devoted his career to her scientific advance- ment and welfare. His many friends and geological colleagues both inside and outside Ethiopia will remember him with love and professional admiration.

D. Adamson, F. M. Dakin and
M. A. J. Williams

Hans-Lukas Teuber

HANS-LUKAS TEUBER, Professor of Psychology at M.I.T., was lost on January 4 while swimming off the British Virgin Islands. It is thought likely that he died of heart disease; he was 60 years old. Many in Britain will share the sense of loss, not only because "Luke" (as he became known in later years as he became fully absorbed into the American culture) was an indefatigable traveller and lecturer, but also because of a memorable year as Eastman Visiting Professor at Oxford in 1971-2. His audience and influence were deservedly world-wide.

In later years he was perhaps best known as the father of a distinguished, indeed unique, centre of psychology and brain sciences at M.I.T., to which his devotion was complete and in which his pride was transparent. But it was his earlier highly penetrating and influential analyses of the problems and mechanisms of visual perception, in particular, and of brain function more generally, with which he first made his mark. His early major

researches were on the psychological analysis of brain wounds in war veterans, carried out while serving with the U.S. Navy during World War II. This type of research remained a con- suming interest until the end, but he contributed a unique and distinctive personal approach to a tradition that had its roots in 19th century neuro- logic. To that tradition Teuber injected a concern for the importance of certain crucial demands that modern psycho- logical analysis places upon the inter- pretation of brain function. He was, for example, one of the first to seize upon the importance of the distinction between externally imposed changes in sensory stimulation and those that result from self-initiated movement, which produces a "corollary dis- charge." He was quick to spot and integrate important modern develop- ments, both technical and intellectual, but these always were seen in a historical context which he viewed with enormous erudition and reverence.

Born in Germany and educated at the French College in Berlin and at the University of Basel, he emigrated to America during the War and

received his doctorate in psychology at Harvard in 1947. He claimed that he was accepted as a desirable emigré, when his ship was stopped at Gibraltar, because he was able to converse freely in Latin and Greek with the young inspecting British officer. His father was founder, for the Prussian Academy of Sciences, of a field station for the observation of chimpanzees on Tenerife, whose fame was later assured by the important work of Wolfgang Köhler (*The Mentality of Apes*) carried out there. This perhaps accounts for Teuber's early interest in problems of visual perception identified by the Gestalt psychologists, and his friendship and admiration for Köhler were life-long.

At Harvard, the story is told, he managed to fail the compulsory German translation because, as a new arrival, he did not know enough English into which to translate the German. But, if so, his command of English soon developed to lofty heights, and he could hold an audience rapt with his eloquent and gently humorous style. In 1947 he joined the faculty of the Bellevue Medical Center of New

York University, where he remained until 1961, when he became Professor of Psychology at M.I.T. His quickness of repartee was renowned. On one occasion, when chided at a symposium by an impatient participant for his frequent references to 19th century authors, Teuber replied without hesitation that he merely wanted to display his originality. And he took a quiet delight in his skill at improvisation and extemporaneous delivery. When a lecture to a large audience in a playhouse was interrupted by a power cut, he continued his lecture, without undue pause, by using his fingers in front of the emergency lamp to project his slides in the form of shadows on the screen.

His move to M.I.T. was at first somewhat turbulent, perhaps because Teuber had a clear and even uncompromising conception of the type of department of psychology he wished to develop. To it he attracted scientists of great distinction from a variety of

disciplines, as well as younger persons whose promise later was fulfilled; the contributions of his colleagues were as important in neuroanatomy and neurophysiology as in experimental psychology. He worked unceasingly to attract funds for their endeavours and to promote a genuinely interdisciplinary atmosphere, warm and paternalistic, in which he and his colleagues could flourish. The M.I.T. department became an almost compulsory stopping-off point in the U.S.A. for scientists from throughout the world with interests in brain function and psychology; they were invariably greeted with great hospitality and kindness, their seminars almost always continuing at the Teubers' home late into the evening, surrounded by a formidable but enthusiastic circle of graduate students.

Ironically, his memorial service was held at M.I.T. on the day on which he was to have received an award given by the Institute to a member of its

faculty for outstanding accomplishment. The citation described him as "a man who joins the instinct of a penetrating experimenter and the sympathetic experience of a true humanist with the consummate style of a gifted teacher." To these qualities may also be added his warmth, skill and dedication as a promoter of neuropsychology both at home and abroad. He was an insightful, popular, and expansive reviewer at international meetings—even as a helpful translator for foreigners, the duration of his commentaries was apt to exceed, by a considerable amount, that occupied by the original speaker. As tragic as was his death, perhaps he would have preferred it to any alternative that might have prevented him from exercising his indefatigable energies at full strength.

He is survived by his wife, Marianne, who was a devoted partner throughout his career, and by two sons, Andreas and Christopher.

A. T. Phillipson

PROFESSOR A. T. PHILLIPSON, who died suddenly on 10 January at the age of 66, was the leading figure in the great post-war revival of interest in the function of ruminant animals. He graduated in agriculture at Cambridge, gained his MRCVS at the Royal Veterinary College, London and then returned to Cambridge to show his flair for research in a Ph.D. study of gastric motility in sheep. Subsequently, he led a small wartime group, under Sir Joseph Barcroft, FRS, at the ARC Unit of Animal Physiology, Cambridge. Between 1941 and 1947 Phillipson, with Dr R. A. McAnally, showed that the hitherto neglected volatile fatty acids are the final end products of microbial digestion of carbohydrates and that they are absorbed through the heavily keratinized epithelium of the rumen into the portal blood in quantities which provide a significant proportion of the energy requirements of the host. Such a radical conclusion was not accepted without controversy, yet attempts by others to find an alternative explanation only reinforced the evidence in its favour. In fact in the last thirty years the conclusions made by this small group have formed the basis for much of the subsequent work on ruminant digestion throughout the world and, when they were extended to other components of the diet, such as protein, completely changed our concept of ruminant nutrition and metabolism. It is salutary to remind ourselves that this major advance was based, after a preparatory period in the late 1930's for thought and observation, on a series of simple and elegant ex-

periments carried out over the relatively short period of about four or five years.

Moving in 1947 to the Rowett Research Institute, of which he became Deputy Director in 1952, Phillipson consolidated the advances made at Cambridge. The years at Aberdeen were marked by special attention to the techniques required for the quantitative assessment of the ruminant digestive processes. Techniques ranging from microbiology to radiography were brought to bear on microbial fermentation and synthesis, ruminal absorption and metabolism of volatile fatty acids, movements of the forestomachs and the propulsion of food to the intestine. He was one of the first to emphasise and measure the extent to which urea may be secreted into the rumen to provide a substrate for microbial protein synthesis. In a prophetic mood in 1947 (*Vet. Rec.* 58(8), 81-85) he had stressed the importance of measuring the amounts of protein and polysaccharide received by the animal in microbial form, of knowing how much starch and protein escapes degradation in the rumen and of assessing the amounts of volatile fatty acids absorbed. In 1977 we are beginning to use such information in evaluating diets.

In addition to his important research, Phillipson wrote a number of lucid reviews outlining the state of knowledge of rumen function, always with exact attributions and a minimum of extrapolation, and pointing the way to further essential investigation. These were required reading for a new generation of animal scientists and must have directed many to the fascination

of the rumen. A remarkably high proportion of the active post-war workers in this field spent time with Phillipson, some to experiment, some just to discuss, but all to be welcomed, involved in his interests and influenced for the future. He travelled a good deal and those he met were also much influenced. In such benign ways, Andrew Phillipson has left a permanent mark on rumen studies. His hallmarks were enthusiasm, wide background knowledge, a preference for direct approaches, accuracy and a habit of being right.

Returning to Cambridge in 1963, Phillipson took the Chair of Veterinary Clinical Studies. Administration and teaching then restricted his opportunities for research, but in these activities he preserved the high standards characteristic of his earlier work. He was chairman of the organising committee of the very successful Third International Symposium on Physiology of Digestion and Metabolism in the Ruminant at Cambridge in 1969.

Phillipson was made FRSE in 1953 and his achievements were recognised by honorary degrees at Copenhagen, Ghent and Edinburgh. In England he received major prizes in agricultural and veterinary fields.

Andrew Phillipson was a charming man whose enthusiasm shone through a diffident manner; an aesthetic, person, especially fond of music, he enjoyed Cambridge and his Fellowship to the full. His family life was extremely happy and the sympathy of many friends, worldwide, will go to Mrs Rachel Phillipson and their three sons.

C. C. Balch